

ON THE SPECIFIC GRAVITY OF THE BLOOD

EXPERIMENTAL EVIDENCE OF DIFFERENCE IN WEIGHT OF THE BLOOD OF THE RIGHT AND LEFT VENTRICLES OF THE HEART

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In consideration of the changes which the blood undergoes in its passage through the lung, the question arises to what extent, if any, is the weight of the blood altered. Is there an exact balance, after the loss of the products of combustion, water, and carbon dioxide, brought about on the intake of oxygen? Or is there want of balance and is the blood of the left side of the heart heavier or lighter than that of the right side? The present paper is the result of an inquiry into this question.

Experiments were conducted on the cat under anaesthesia. Healthy, adult, fasting animals of both sexes were used. General anaesthesia was obtained in one hour after administration of a 20 per cent solution of eurethane by the mouth in the dose of 7 cc. per kg. of body weight. Blood for testing was drawn by means of hypodermic needles from the left ventricle or common carotid artery and from the right ventricle by methods which are described below in detail.

Regarding the method for finding the specific gravity of blood, the older procedures of Jones¹ and of Hammerschlag² were first tried and abandoned because not sufficiently delicate for our purpose. We found the falling-drop method of

¹ Jones, E. L. 1887 On the variations in the specific gravity of the blood in health. *Journ. Physiol.*, vol. 8, p. 1.

——— 1891 Further observations on the specific gravity of the blood in health and disease. *Ibid.*, vol. 12, p. 298.

² Hammerschlag, A. 1892 Eine neue Methode zur Bestimmung des specifischen Gewichts des Blutes. *Zeitschr. f. klin. Med.*, Bd. 20, S. 444.

Barbour and Hamilton³ reliable and exceedingly sensitive to variations in specific gravity. Our experience has taught us that speed in making the test is essential for obtaining constant results: delay introduces the tendency to clotting and exposes the blood to the atmosphere, both of which affect the weight of the drop to be tested. Withdrawing a drop of living blood from an artery or from the heart directly into the pipette gave satisfactory results. It is apparent that data on the specific gravity of the blood, and more especially of the arterial blood, are worthless for a normal record if to any considerable extent the blood pressure, heart action, and respiration are affected; therefore, it became necessary to devise methods adapted to safeguarding these functions.

THE EXPERIMENT

We have found that the experiment, which demands quick action and involves two different sorts of work, can best be done by two workers; in the following account they will be referred to as operator 1, and operator 2. In general, operator 1 performs the vivisection; operator 2 makes the blood test.

An adult cat, anaesthetized by means of eurethane, is placed dorsicumbent on an animal board which has been rigidly fastened across a narrow laboratory table and so inclined that with one end resting on the table, the opposite end, supporting the cat's head, will be raised to the level of the shoulders of operator 1, who stands while performing the experiment. Tracheotomy is advisable in the event that artificial respiration may become necessary. Both common carotids are exposed and carefully freed from the accompanying vagi. The left carotid (the femoral artery is preferable in a large cat) is connected with a mercury manometer for recording pulse and blood pressure; a pneumograph is

³ Barbour, H. G., and Hamilton, W. F. 1924 Blood specific gravity: Its significance and a new method for its determination. *Am. Journ. Physiol.*, vol. 69, p. 654.

1926 The falling drop method for determining specific gravity. *Journ. Biol. Chem.*, vol. 69, p. 625.

belted to the thoraco-abdominal zone and connected with a tambour to record the respiratory movements. An initial record is then made, and if all is well the test proceeds. Continuous records for the experiment have proved that only slight temporary effects have occurred in respiration and blood pressure in the course of the experiments.

The blood of the right side of the heart is tested first. A drop is taken directly from the right ventricle, which is approached via the cephalic (superior) mediastinum. The operation calls for simple blunt dissection by means of a scalpel handle, entering the cephalic aperture of the thorax and pushing ventrally and laterally the two layers of mediastinal pleura until the pericardium has been reached. As a preliminary measure, strong ligatures are passed by a needle through the sternohyoid and sternothyroid muscles 1 cm. cephalad of their origins from the first ribs, then through the cut edges of the skin, drawn laterally and ventrally, pulled taut, and tied to a frame made for the purpose and fastened to the animal board. By means of the ligatures, the entrance to the mediastinal tunnel is kept open. As the heart is approached, a long narrow nasal speculum is inserted and carefully opened laterally, holding the laminae mediastinales apart sufficiently to afford working room without undue compression of the lungs. A bright beam of light from an arc lamp is directed to the bottom of the tunnel by means of a head mirror. In opening the tunnel, the dissection is begun and is continued upon the ventral aspect of the trachea, which is finally exposed almost to the bifurcation. The carotids, innominate, and precava can be identified in the ventral wall of the tunnel near the heart. Shining through the pericardium at the bottom of the tunnel can be seen the termination of the precava as a distinct dark purple-colored area and, less clearly, the eminence of the aortic arch. The tunneling operation occupies from thirty seconds to one minute, is bloodless, and free of danger if carried out as described.

Tapping the right ventricle

The instrument. A specially prepared Luer slip-on needle is used. The 2-inch, 18-gauge needle was found best; smaller diameters favor clotting in the lumen. A shoulder was provided by soldering a wire ring around the shaft 27 mm. from the tip; this is to prevent too deep penetration through the heart and so beyond the site of the ventricle. Upon one side of the slip-joint of the needle a slender, but stiff, steel rod, 13 cm. long, is soldered, extending backward parallel to the axis of the needle. The distal end of the Barbour pipette was specially ground to fit the Luer needle. When the thrust is to be made, the needle is slipped onto the end of the pipette, the steel rod lying alongside. The joint is made air tight by smearing the ground surface with a heavy inert grease. The pipette and rod are grasped together in making the thrust into the ventricle, and with this provision there is no danger of uncoupling the needle in the manipulations that may be necessary. After the needle has penetrated the heart and the pipette filled with blood, the latter can be uncoupled with one hand and withdrawn by holding the steel rod with the other hand and retaining the needle in the heart.

The operation. In preparation for the tapping, pipette and needle must be cleaned and dried with scrupulous care; washed in water, followed by alcohol, and the latter removed with ether, then dried by an air current. The pipette furnished with a rubber tube and coupled with the needle is directed by operator 1 down the cephalic mediastinum, and when the heart, well lighted, is in view, the point of the needle is driven through the pericardium at a spot midway between the termination of the precava and aortic eminence and about 1 cm. ventral to the trachea. The direction of the thrust is caudad with a very slight inclination ventrad. The thrust is continued until arrested by the shoulder of the needle meeting the pericardium.

Just before making the thrust, the rubber tube attached to the pipette has been closed with a bulldog clamp to prevent air entering the right atrium during the passage of the needle

through it and also to prevent blood from entering the pipette before operator 2 is prepared to control the column. In making the thrust, operator 1 observes the degree of resistance encountered and, when the needle is in the heart, notes the amount of oscillation which the pipette undergoes. Regarding the resistance, if great enough to stop the needle, the point has probably met the aortic arch, and if the vessel has been entered the hemorrhage will terminate the experiment. If the resistance is barely perceptible, the needle will have probably entered the right atrium and penetrated the right ventricle via the right venous ostium. When the resistance is moderate, the point of the needle has been found to have engaged the right ventricular wall at a spot between the right auricle and ascending aorta and to have gone onward to enter the right ventricle. This is the best route, for the point of the needle in the conus arteriosus is away from the segments of the tricuspid valve, the trabeculae cordis, and chordae tendineae, all of which interfere with the free entrance of blood into the lumen of the needle. In regard to the sign of oscillation: when vigorous, the needle has been found in the ventricular cavities or their walls; if slight, in the postcava. It has been noted that the oscillatory movements are not very marked when the needle has found its way into the right ventricle via the ostium venosum.

When the thrust has been made and the indications are that the point of the needle is free in the right ventricle, then the rubber tube is taken in the lips of operator 2 and compressed and the bulldog clamp released. Blood may flow into the pipette at once on relieving the compression on the tube, or it may be necessary to start the flow by suction. If blood does not come after two or three attempts, it is probably because the needle is in the heart wall; the instrument should be withdrawn, washed out, dried thoroughly, and another thrust made. If fluid enters the pipette, the operator must observe its color and be on the watch for bubbles caused by air entering a faulty coupling of the needle and pipette. A small quantity of straw-colored pericardial fluid may precede

a column of blood, having entered as the point of the needle traversed the pericardial cavity before it pierced the ventricle, or watery fluid alone rises in the lumen, proving that the point of the needle is not properly located. These conditions and the presence of air will, of course, necessitate retrieval. Needle and pipette are withdrawn, thoroughly washed out and dried, and the thrust repeated. When a column of blood is obtained free of extraneous fluid and bubbles, operator 2 seizes the steel rod with his left hand and steadies the needle, while with his right hand he carefully uncouples the pipette from the needle and withdraws it, leaving the needle undisturbed in place in the heart. Then with all speed possible the blood column is measured off in the pipette, the drop of blood released in the tube containing the XBB mixture and its falling time observed in accordance with the method recommended by Barbour and Hamilton for determining the specific gravity. While the blood is still in the pipette, the color, whether bright or dark, is observed.

Obtaining arterial blood. During the period when the second operator is observing the falling time of the drop of venous blood, the first operator makes ready for taking a sample of arterial blood from the right common carotid artery. First, however, a small plug of cotton is placed in the cephalic (superior) mediastinum, in order to prevent the needle from slipping out of place; the speculum is then removed. Immediately upon finishing his record of the falling time of the venous drop, operator 2 cleans the pipette and fits to it a finer Luer needle (1-inch, 23-gauge). Obtaining arterial blood is a very simple procedure: the right common carotid artery is supported by the first operator while the needle mounted on the pipette and held by the second operator is pushed through the coats into the lumen of the vessel. The rush of blood into the pipette is checked by compressing the rubber tube and withdrawing the needle. The slight hemorrhage resulting from the needle wound should be controlled by partial pressure of the artery with the fingers; complete compression should be avoided. In order to obviate

the closure of both carotids at the same time, the manometer was connected with the femoral artery in some of the experiments, or the arterial blood was obtained from the femoral—we found the carotids more favorable to success. On securing a column of blood, the test of the falling drop is made with all expediency. In order to have comparable data, the time elapsing between the two tests, that for the blood of the right ventricle and that for the common carotid, should be reduced to the utmost minimum. Practice and a steady hand will shorten the time to two minutes or less.

At the conclusion of the experiment, the animal has been sacrificed (chloroform) and an autopsy performed chiefly for the purpose of locating the needle. The method adopted consists in opening the chest by removing the ventral thoracic wall and inspecting the lungs for possible wounds; completing the opening of the mediastinum from the ventral aspect and noting the position of the base of the needle; slitting the pericardium and observing the presence or absence of blood; demonstrating the route of the needle and the position of its point. If the latter is not in the right ventricle, the tests are, of course, useless. If the needle is in the postcava the result should not be used. Blood in the postcava differs in quality (portal and renal inflow, etc.) from that in the precava (lymph and chyle inflow), and the two have probably varying specific weights. Complete mixture of these bloods probably does not take place until the charge has passed through the rough-walled ventricle during systole into the smooth-walled conus and pulmonary ostium.

RESULTS

The results of the experiments are shown in tables 1 and 2. The data given in table 1 are specific-gravity records obtained from the blood of living animals by several different methods. Methods I and II were found not always dependable and were eventually abandoned in favor of method III. Averages of results and also figures which appear to indicate correlation of specific gravity of the blood with sex are shown in table 2.

DISCUSSION

On the method of obtaining samples of blood

In the earlier experiments arterial blood was taken from the carotid or femoral artery after tying the vessel, applying a clamp 1.5 cm. proximal, nicking the coats, and then, by releasing the clamp, allowing a small amount of blood to flow into a paraffined porcelain cupelle. Two tests at most could be made, using one set of instruments, before clotting occurred. The results were not consistent. Exposure of the

TABLE 1

Determinations of the specific gravity of the blood of the right ventricle and of arterial blood in the cat

METHOD	DATE	SEX	BLOOD OF RIGHT VENTRICLE	ARTERIAL BLOOD	INCREASE
I. Cannula via jugular vein	2-21-27	F	1.0527	1.0534	.0007
	2-23-27-A	F	1.0486	1.0506	.0020
	-B	F	1.0520	1.0525	.0005
II. Thorax opened and heart penetrated di- rectly	2-19-27	F	1.0491	1.0515	.0024
	2-25-27-A	F	1.0465	1.0489	.0024
	-B	F	1.0481	1.0507	.0026
	2-26-26-B	F	1.0499	1.0523	.0024
	3- 2-27-A	F	1.0489	1.0495	.0006
	3-31-27-A	M	1.0492	1.0513	.0021
	-B	M	1.0487	1.0498	.0011
	-C	M	1.0514	1.0529	.0015
	4- 1-27	—	1.0506	1.0532	.0026
	5- 5-27	M	1.0473	1.0511	.0038
	6- 9-27	M	1.0507	1.0530	.0023
	6-10-27	F	1.0477	1.0483	.0006
	6-13-27	F	1.0424	1.0431	.0007
	6-14-27	F	1.0480	1.0483	.0003
	6-15-27	M	1.0451	1.0456	.0005
	6-16-27	M	1.0430	1.0454	.0024
	6-17-27	M	1.0525	1.0532	.0007
III. Superior mediastinal route	6-20-27	M	1.0521	1.0545	.0024
	6-22-27	F	1.0484	1.0508	.0024
	6-24-27	M	1.0481	1.0518	.0037
	6-27-27	M	1.0510	1.0516	.0006
	6-28-27-B	F	1.0471	1.0478	.0007

blood to the air was eliminated by first dispensing with the cupelle and collecting the blood directly with the end of the pipette from the opening in the artery; later, by the hypodermic-needle method already described. Finally, tying and clamping the artery were abandoned, and so the possibility of changes in the blood that might step in from injury to the arterial coats was greatly reduced, if not completely eliminated. Arterial blood was taken directly from the left ven-

TABLE 2
Averages of results

	NUMBER OF EXPERIMENTS	SPECIFIC GRAVITY			
		Right ventricle blood	Arterial blood	Difference	Per cent gain
Method I	3	1.0511	1.0522	0.0011	0.10
Method II	17	1.0482	1.0499	0.0017	0.16
Method III	5	1.0493	1.0513	0.0020	0.19
Combined results of I, II, III	25	1.0488	1.0504	0.0016	0.15
Sex in relation to specific gravity (data from methods I, II, III)					
Male	11	1.0490	1.0509	0.0019	0.18
Female	13	1.0484	1.0498	0.0014	0.13

tricle in the series of experiments in which the thoracic cavity was opened. This method will be referred to below.

The technic of obtaining blood from the right ventricle was a more difficult problem. Three methods were developed and tried: 1) via the jugular vein; 2) by opening the thorax; 3) via the cephalic (superior) mediastinum.

1. Many fruitless trials were made to perfect a method by which a drop of blood could be withdrawn from the right ventricle by an approach through the veins. A cannula was directed down the right jugular vein: *a*) frequently blood would clot in the former before it could be sucked out, or, *b*) air would enter the circulation. Introduction of a cannula

through the right jugular route will probably interfere with the normal entrance of lymph from the right side and will certainly disturb the return of venous blood from the right side of the head and upper limb. Trocar and cannula penetrating the precava in the cephalic mediastinum was a procedure attended with objection *b*, and was not continued. Entrance via the postcava (abdomen opened) can be made by means of a cannula having a shepherd's-crook end which, after reaching the right atrium, can be pulled caudad into the right ventricle. Clotting in the cannula occurred; there is also the question of interference with the return blood to be considered.

2. Blood was drawn by a needle from the right ventricle after opening the thorax. Artificial respiration was maintained, but the results of the tests for the specific gravity, while consistently showing the tendency confirmed by later experiments, were not entirely satisfactory, because of difficulty in preserving the blood pressure and maintaining normal respiration. In some of these experiments the arterial blood was taken from the carotid artery prior to testing the blood of the right ventricle; in others, from the left ventricle. In the latter case the discrepancy between the weights in the bloods of the two sides of the heart was demonstrated whether the right side was the first tested or whether it was the left. We mention this because, in these experiments of opening the thorax, the specific gravity of the left blood tended to approach that of the right after a number of tests were made; the first test showed consistently that the left blood was heavier than the right. To open the thoracic cavity the pectoral muscles were separated from their thoracic origins and turned laterally. A scissors cut was made 1 cm. to the left of the sternum through all costal arches as far as the diaphragm, care being taken not to injure the right mediastinal pleura. Hemorrhage was controlled by long-bladed clamps compressing the two cut margins of the chest wall. In preparation, tracheotomy was performed and air introduced under sufficient pressure. After the opening into the

thoracic cavity was made, the pressure was adjusted to keep the left lung normally inflated, but the movements of the lung were not satisfactorily brought about. The pericardium was slit open, the heart seized at the apex with forceps or held by the fingers when the Luer needle was inserted. The latter, mounted on a Barbour pipette, was pushed into the right ventricle through the conus arteriosus and into the left ventricle through the apex.

3. All of the earlier methods to obtain the blood of the right ventricle were abandoned in favor of the approach by the mediastinal route described above. Although by this method two or three trials may be necessary before a successful thrust is made, it has the advantage over the other methods tried in not disturbing relations and functions to any considerable extent, in that it is rapid and that the source of the blood drawn can be demonstrated beyond question.

*The general tendency of the specific gravity of the blood
of the right and left sides of the heart*

One significant fact brought out by the figures in table 1 is the tendency shown for the arterial blood to be heavier than the blood of the right ventricle. The results of the tests by the three methods employed, all show this tendency. The figures of the less perfect methods I and II, while presenting considerable variation in the amount of increase, nevertheless give evidence without exception of some gain in specific gravity of the blood after having passed through the lung. The increase, as the figures show, is slight, but it is a constant phenomenon. If this slight difference between the two bloods included now and then an instance in our record of higher specific gravity on the right than on the left, it might well be questioned whether our results were not influenced by imperfections in the method, and that if, with greater care, it might not eventually be shown that the weights were the same on the two sides. The constancy of the results leads us to conclude that the left blood is heavier than the right under the conditions of the experiment.

On the weight of evidence from method III

The preparation of the animal for this experiment is attended with little risk of introducing elements which would seriously affect the results. Table 1 shows that five tests of the specific gravity were made by the mediastinal route, and all give evidence of an increase in the density of the blood after passing through the lung. It will be remarked that there is a wide range between the increase in specific gravity as shown in the first three experiments and that of the last two (dated 6-20, 6-22, 6-24, and 6-27, 6-28); such a range is presented also in the figures of methods I and II. We consider these variations in results as probably indicative of natural variations in the conditions of the respiratory and circulatory mechanisms rather than errors in the control of the experiment. Our experience with method III induces us to regard its results as sound and reliable.

On evidence of a correlation between sex and the specific gravity of the blood

A number of observations have been published showing a definite correlation between sex and blood density. The figures in table 2 are derived from twenty-four experiments, eleven on male and thirteen on female animals. Although the material is too limited to warrant a conclusion, it is evident that the figures for both the arterial blood and the blood of the right ventricle represent a higher specific gravity for males. The gain in density of the arterial blood appears absolutely greater for males than females, according to these figures; whether it is relatively greater is a question that must wait upon the testing of a larger number of cases. The figures of table 1 show a greater range of variation in the female, both in the density of the arterial blood and in the blood of the right ventricle.

*On the range in variation of specific gravity of the blood
of the right ventricle and of arterial blood*

Because of the qualitative variations in venous blood, it was our expectation that the density of the blood of the right ventricle would tend to vary considerably and that the specific weight of the left ventricular blood would show a certain stability in contrast. The figures of table 1 do not support this expectation; indeed, they show a slightly greater range of variation for the arterial blood. The range is 0.0103 for the venous side and 0.0114 for the arterial. Apparently, the balance between the loss of combustion products and gain in oxygen as the blood circulates in the lung fluctuates within a considerable range of normal limits.

*Speculation as to the factors determining the difference in
specific gravity of the blood of the right and left sides
of the heart*

The lung excretes the products of combustion, water, and carbonic acid and exposes the blood in its capillaries to a recharge of oxygen. The blood loses a volatile element, carbon dioxide, by which its specific weight should rise slightly; but such increase in density would be neutralized by the intake of oxygen when the respiratory quotient is unity. Although the animals in the present experiment were not on a carbohydrate diet, they were fasting, and it is necessary therefore to withhold any conclusion as to the factors which cause the change in the density of the blood until work now in progress can be completed. The extraction of water from the venous blood on entering the lung capillaries and its escape in the expired air must be taken into consideration as a factor in bringing about the rise of the specific gravity of the blood as it reaches the left side of the heart.

SUMMARY

In attempting to answer the question as to what change in weight of the blood, if any, takes place during its passage

through the pulmonary vessels an experiment was planned and developed by which samples of blood were obtained from the right and left sides of the heart of the cat. These samples, protected from contamination by other fluids and from exposure to the air, were rapidly tested for specific gravity by a reliable and delicate method. The results throughout indicated a difference in specific gravity of the right and left blood and that the blood gained in density in passing through the lungs.

CONCLUSION

In the fasting cat under eurethane anaesthesia, with blood pressure, pulse, and respiration normal, the blood of the common carotid artery, and by inference of the left ventricle, is denser than the blood of the right ventricle.

DESCRIPTION OF A YOUNG HUMAN ANENCEPHALIC AND AMYELIC EMBRYO

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SEVEN FIGURES

The monstium described in this paper is the result of the only pregnancy of a Chinese woman, who denies the occurrence of other deformities in her family. Her Wassermann reaction was negative. The last menstruation occurred one hundred days before the abortion, at which the closed chorionic sac was discharged. The ovum was fixed in toto in formalin, and kept in that condition for over a year. On opening the sac, it contained a 23-mm. embryo with the deformities which form the subject of our description. Paraffin sections, 10 μ thick, were stained with haematoxylin and eosin; they were cut at right angles to the back of the embryo.

GENERAL DESCRIPTION

The amnion sac is 10 cm. long and nearly cylindrical in shape, 4 cm. in diameter. As mentioned already, the embryo itself measures 23 mm., vertex-breech length, but, due to the abnormal flexures and the partial anencephaly, this length cannot directly be compared with the length of normal embryos. On inspection (fig. 1), we see that the base of the skull is covered by a brown mass, which is separated by a sharp border-line from the white skin which covers the face and forehead. The head has not the protruding eyes of the full-term anencephalic monster, and the forehead is present, although it slopes backward instead of bulging over the face. The above-mentioned brown mass contains the cerebral hemi-

spheres and the optic thalamus, which, as we shall see later, is all that is present of the central nervous system. In the center we find an opening with torn edges leading into the brain ventricles.

The thoracic part of the back is covered by skin; it is flattened out, and has a groove in the center where the spinal cord and the arches of the vertebrae ought to be. Downward, this groove leads into a canal which will show itself to

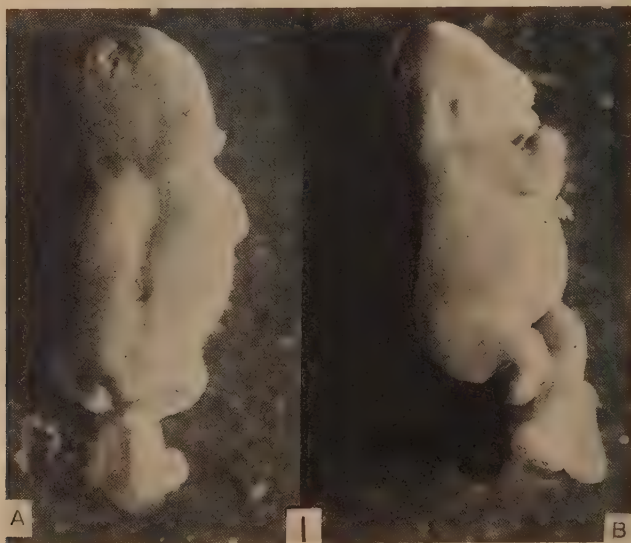


Fig. 1 Side view and back view of the embryo.

be the meningeal sac, and can be followed into the tip of the sacral region.

The embryo has a straight back. The normal kyphotic flexure of the whole vertebral column from sacrum to neck is absent. The head is not bent forward, but a little upward.

The umbilical cord is rather short, 2 cm. in total length. It shows a swelling near the abdominal end, which will be discussed afterward. The skin is not intact everywhere, but in some places strings of tissue are attached to it. These

are possibly amniotic bands, but no connection with the wide amnion has been found.

Age of the embryo

As mentioned above, from menstrual data the age must be between seventy-two and one hundred days. The amnion sac of 10 cm. is as large as that of a three-months embryo, but our embryo itself is considerably smaller. The length, 23 mm., corresponds with eight weeks. The stage of ossification of the clavicles and of the long bones of the arms and legs, and the absence of ossification in the vertebrae also point to an age of eight weeks. Yet the end phalanges of the fingers are already cartilaginous—a stage, which, according to the text-books of embryology, does not begin before the third month.

The configuration of the mouth is, as far as I can ascertain, normal for the length of the embryo. There are erythrocytes without nuclei, profusely in symmetrical places, lateral to the central nervous system, and much less clear in the larger blood vessels or in the heart, where they have probably disappeared through hemolysis.

The development of those parts of the brain that are actually present is certainly not delayed. So far as can be inferred from material available for comparison, the brain cortex and the fiber systems of the internal capsule in this embryo are approximately the same as in a normal 27-mm. human embryo.

My conclusion as to the age of our specimen is that the ovum is about twelve weeks old, but that the embryo shows a retardation in its development. Mall accepted the same explanation for many of his cases which also showed an amnion several weeks older than the embryo within. The embryo has an average age of eight weeks, some parts, as the hands and perhaps the hemispheres of the brain, being more advanced in development. It is interesting to note, in this connection, that Nañagas found, in comparing the body dimensions of anencephalics and normal fetuses, that the for-

mer invariably had much longer arms, and that other parts of the body also were not harmoniously developed.

Preservation of the embryo

The preservation of the embryo was not very good. Throughout the body the mucous membranes are badly preserved, their cells lying in groups in the cavities of the organs. The brain also is not well preserved, its structure is often obscured by postmortem disintegration. The cells of liver and heart do not take nuclear stains, but the lungs and kidneys are much better preserved. Red blood corpuscles are found especially well preserved under the dorsal covering of the head. In most blood vessels and in the heart itself they cannot be distinguished easily. On the other hand, it is of interest that the brain and retina show a great number of mitotic cell divisions, and that these occur also, to a less degree, however, in the kidneys and tongue muscle. This must mean that the death of the embryo occurred abruptly. We are led, therefore, to believe that the embryo developed at a slower rate than the amnion sac, but died at the same moment as the latter.

Other deformities

There are a certain number of tags on the skin, especially around the mouth, on the arms, and on the thorax. The epidermis is absent in these parts, and a thin tissue is attached to the skin, covered by irregular connective-tissue cells. There is no evidence of a connection of these structures with the amnion, but it is possible, of course, that such a connection existed at an earlier period.

The umbilical cord contains a tumor-like mass, which on section turns out to be a gland-like structure, connected with the omphalo-enteric duct. It is not within the scope of this article to deal fully with the particulars, but it is interesting to note that a number of mitotic cell divisions were seen in the epithelial cell layers.

The abnormal flexures of the back and neck are the same as seen in the majority of cases of anencephaly and amyelia. The wall of the hemispheres and the retina show abnormal foldings, in consequence of which their cavities are nearly filled up. This phenomenon is often seen in poorly preserved embryos, whether otherwise normal or not. As far as I know, no satisfactory explanation of it has been given.

Little need be said about the non-nervous organs. With the exception of the epiphysis, of which no trace could be found, all other organs are present. The hypophysis is large, at least in its glandular part, and has a connection with the infundibulum. The adrenals are present, but, due to the bad preservation of the specimen, I was not able to distinguish between its cortex and marrow. The choroid plexus in each lateral ventricle is large and fills up a considerable part of the ventricle. The third ventricle at this period of development still lacks a plexus, and the plexus of the fourth ventricle together with the whole hindbrain is undeveloped.

As far as the state of preservation permits us to judge, the muscles are well developed. There is no difference in development between the eyeball muscles, tongue, masseters, and muscles of the extremities.

The membrane covering the head and neck, and the meninges

As the covering of the nervous defect may be a guide to the origin of this deformity, I will give a detailed description of the various structures found. We can divide the whole into three fields—the head, the thoracic and the lumbosacral portion. For all three fields we can state that the skin, the epidermis as well as the corium, is not continuous with any of the structures to be mentioned. Wherever skin covering and another membrane come together, there is a sharp border-line, as if cut with a knife, and, as a rule, the skin forms a wall several cell layers thick. The normal skin runs above the eyes backward for a certain distance, more than is usual in full-term anencephalics (fig. 1). This is bordered in the frontal and the parietal region by a layer of cubic epithelial

cells, one cell thick. This cubic cell layer is of horseshoe shape, and therefore does not cover the whole defect, and does not border the skin of the neck. It often shows verrucous growths; the normal corium, the fibrous layer present in normal skin, is absent in the whole area, but in some places there is a dense subepithelial tissue in which occur many erythrocytes (possibly hemorrhages).

The remaining part of the head area is either covered by a plate cell, probably connective-tissue scar, or by larger cells, lying scattered on the former, and whose nature cannot be determined, but sometimes resembles ependyma cells. It is possible that these cells, in an earlier period, formed a continuous layer. At the opening in the covering, at the vertex, the meninges are continuous with the mesodermal layer. It is therefore also possible that the larger cells were continuous with the ependyma of the third ventricle, but no proof for this suggestion can be found, as these tissues are all badly damaged. Meninges around the brain are present, although not everywhere differentiated to the same degree. They follow the larger fissures in the lateral aspect of the hemispheres and also lie between both hemispheres and the thalamus.

In the thoracic part (fig. 2) of the embryo the skin is continuous over the back. There is no indication in the midline that an original defect in that position has been secondarily covered by an extension of the epidermis from both sides. There is no sign of meninges under the skin, but mesodermal tissue, of course, is present. The spinal ganglia have no distinct membrane, no meningeal cavity is present, such as is found in the sacral part.

At the lower thoracic level the epidermis splits in the median line, and this gap becomes gradually wider as it proceeds caudally, the floor of the groove being covered by mesodermal tissue. Gradually the groove becomes deeper, and finally the walls close again in the median line. Then a tube is formed, lined by mesodermal tissue, the meninges, and the surface is covered again by normal epidermis (fig. 3).

This meningeal sac of the lumbosacral region shows an incomplete division into two halves at several levels, caused by a septum projecting from the anterior wall into the cavity. This is very probably the anterior septum of the pia project-

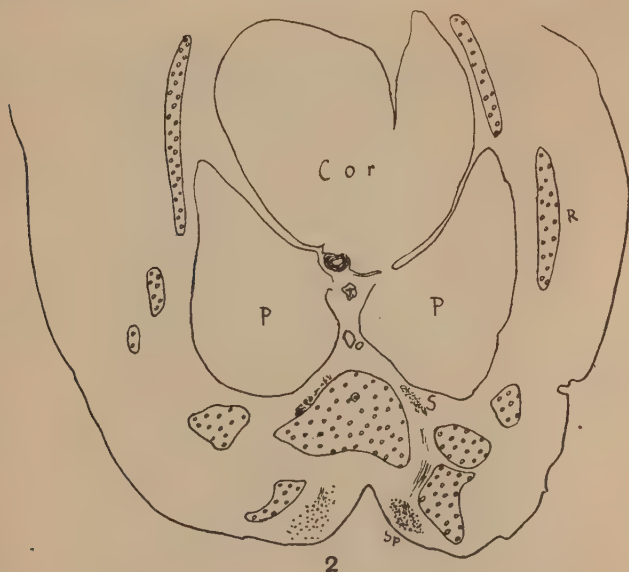


Fig. 2 Diagram of cross-section of upper thoracic part of the body. Note the spinal ganglia (*sp.*, of which the right is much more dense than the left) on both sides of the groove in the skin. No meninges present. Note the sympathetic ganglia (*S*) on both sides of the body of a vertebra, and some fibers arising from them (white communicating branch). The vagus nerve, on both sides of the oesophagus (*O*) is not drawn. *P*, lungs; *T*, trachea; *R*, rib.

ing into the anterior fissure of the spinal cord—an assumption which is corroborated by the existence in some places of spinal-cord tissue within the sac. The spinal ganglia of the lumbar portion lie lateral to the meninges. In the sacral part they are attached to the pia. A dura does not exist as a separate membrane.

Central nervous system

The central nervous system of this embryo, therefore, consists of both hemispheres and the thalamus at one end, and some remains of lumbosacral spinal cord at the other end. In the segments between these two parts, only spinal ganglia and some cranial nerves are present.

The cerebral hemispheres are not much deformed. Owing to the deformation of the whole head, their position is more

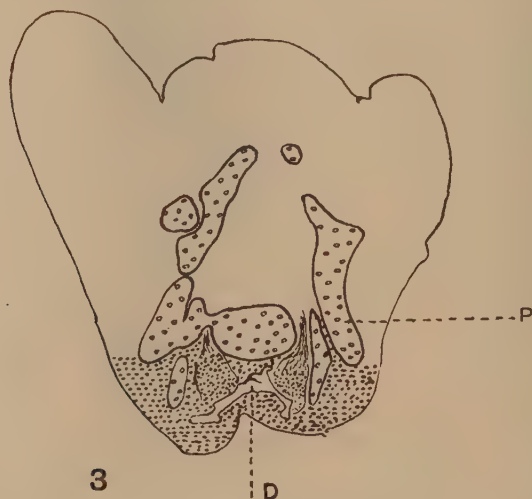


Fig. 3. Diagram of cross-section through the lumbosacral part. The spinal ganglia lie laterally of the empty meningeal sac (fallen together by shrinkage). The organs in the pelvis not drawn. *P*, pelvis; *D*, meningeal sac.

backward than in the normal embryo, but their size is probably normal. As stated above, the wall of the vesicle is often folded. One fissure runs on the lateral side from the top of the hemisphere downward and can be compared with the Sylvian fissure, as it is around this fissure that we find the anlage of the striate body (fig. 4, *BG*.) It is also in this part that we find a primitive cortex (*C*) of the same distribution as in slightly older normal embryos. It spreads only over the lateral aspect of the hemisphere. The choroid plexus is

rather massive and is filled with dense connective tissue; its ependymal cell layer can often be distinguished in parts where the postmortem maceration is not too severe. The olfactory lobes and nerves are present; the foramen of Monro is rather large.



Fig. 4 Photograph of a section through the brain, at the level of the opening on top of the skull (between *x—x*), and through the foramen Monroi. Note the large basal ganglia (*B.G.*) with cortex formation (*C*) on their surface. Note choroid plexus (*C.P.*) in both lateral ventricles. The ventricle of the thalamus seems to open at the back.

Between both hemispheres lies a part of the brain that, I think, must be the optic thalamus (fig. 4, *th*). The basal structures are quite typical. As shown on the accompanying diagram (fig. 5), the optic nerves and chiasma are distinctly developed. But an optic tract cannot be found as a compact fiber bundle. The fibers from the chiasma cannot be traced caudally of their crossing for any considerable distance. The

third ventricle opens into the foramen of Monro in the lower part of this communication between the two hemispheres. From there backward the roof of the thalamus is not membranous, but rather thick. This organ itself is a rather massive structure, with a small ventricle, which, however, has

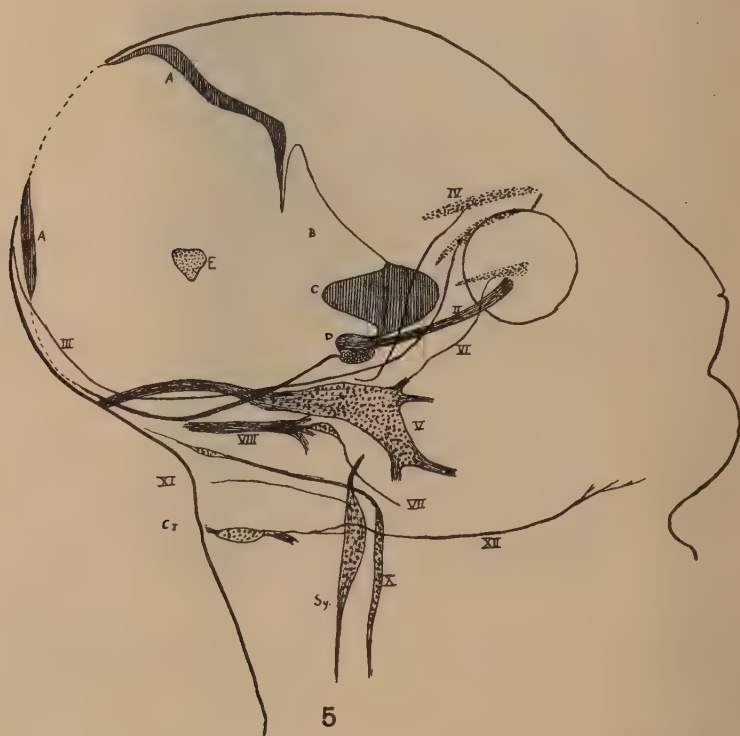


Fig. 5 Diagram showing projection of the thalamus and cranial nerves on a sagittal plane. *A*, median section of thalamus; *B*, foramen Monroi; *C*, septum pellucidum; *D*, chiasma, below which lies the hypophysis; *E*, place where the internal capsule perforates from the striate nucleus into the thalamus. Roman figures *II* to *XII*, the cranial nerves; *CI*, first cervical nerve. *Sy*, sympathetic nerve and superior cervical ganglion. The three eye muscles are the *m. trochlearis*, *m. rect. sup.*, and *m. rect. ext.* The *V* and *VII* nerves have not been drawn to their peripheral termination. The gasserian ganglion and the surrounding nerves are drawn in the diagram more caudally, in order not to get their projection covering the chiasma.

two lateral processes leading into lateral masses of nervous tissue, of which I cannot find the homologue in the normal embryo. The roof of the thalamus is discontinued in two places; one is the opening in the covering of the nervous system, where the ventricle opens into the amnion cavity. Owing to the bad preservation of the embryo, I have not been able to determine whether the ependyma is continuous with the covering or not. The second opening lies more backward and is covered by mesodermal tissue. It is possible that an ependymal roof covered the ventricle here, but was macerated after the death of the embryo.

The internal capsule can be seen in the diagram (fig. 5, *E*). Fiber tracts run from the primitive cortex and from the striate nucleus into the thalamus, where a part of them bends upward (striothalamic tract) and another part runs backward (corticostriomesencephalic tract). This distribution of the fibers and the direction of the tractus habenularis, which is clearly present, make me feel sure that all, or by far the larger part, of this nervous ganglion is thalamus, and not mesencephalon.¹ In the normal embryo these parts are pressed in a frontal direction by the mesencephalon and by the pons, which lies very close to the optic chiasma.

I cannot determine which part of the thalamus, as we see it now, was connected with the mesencephalon, but probably it must have been the lower caudal opening. When we admit a destruction of the mesencephalon and all more caudal nervous structures, the extreme posterior position of the hemispheres and the thalamus is easily explained by a pressure on the frontal region without any counterresistance at the back of the head.

The spinal cord is absent in the cervical and thoracic region, where no meningeal cavity exists. No motor roots can be found, but the connecting branches from the spinal ganglia to the sympathetic ganglia are present. However, as far as I know, the point is not yet settled whether these nerves

¹ We will see later that the distribution of the cranial nerves also is in favor of this conclusion.

carry only motor fibers, whose cells lie in the lateral part of the anterior horn, or also sensory fibers from the internal organs, going to the spinal ganglion. The presence of these communicating nerves does not imply the presence of motor fibers in this part of the embryo. Searching for motor end plates in the muscles of the extremities was not practicable,

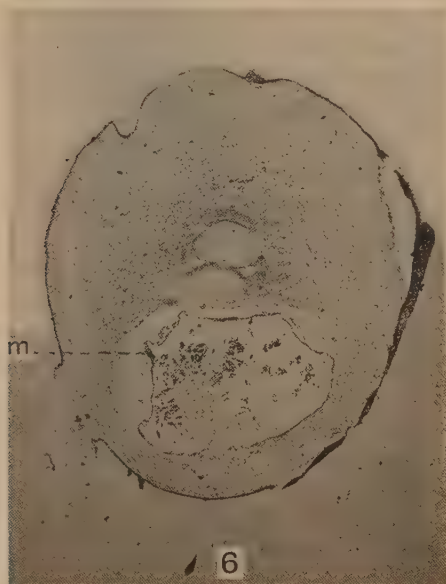


Fig. 6 Photograph of lower sacral vertebral canal. In the meningeal sac (*M*) are remnants of nervous tissue, in which various elements of the embryonal cord can be distinguished. No spinal ganglia present in this section.

because the fixation of the tissues was insufficient for the appropriate staining methods.

In the lumbosacral part we find a meningeal sac (fig. 6), and in this sac some scattered nervous tissue can be found in places. Often neuroblasts and embryonal white matter can be clearly distinguished, but the particles of tissue are mostly very small and are for long stretches entirely absent.

Sometimes this nervous tissue is connected with the pia, but in most places it lies free in the cavity. I nowhere found a connection with the places where the posterior or anterior root would, in normal cases, perforate the meninges. In this part, as in the thoracic, spinal ganglia are beautifully developed, and their nerves run through the intervertebral foramina, giving branches to the sympathetic ganglia. From the ganglia root fibers can often be traced for a short distance, running in a caudal direction, without any further connection with the pia or the skin. They do not run upward as is described in older cases of amyelia. No anterior root fibers are present.

The spinal ganglia still lie in the groove of the vertebrae; not so far lateral that they lie in the interspinal canal; they form a continuous row, but each is distinct from the others. Their ganglion cells are numerous and well developed; the ventral cells being, as a rule, larger than the dorsal cells.

I have not been able to make out whether or not there are motor nerve fibers in the periphery of the body. As we know, a so-called motor nerve always carries, in addition to the motor fibers, the sensory fibers coming from the muscle spindles, and is therefore always a mixed nerve. This probably also is true for the phrenic nerve, present as a very thin bundle in my case, which, however, carries also some sympathetic fibers.

Cranial nerves

The olfactory and optic nerves are present, and have normal connections with their sense organs, and the olfactory nerve also with the olfactory lobe of the brain. The optic nerves form a chiasma, as described above, but their fibers disappear behind this in the general indistinct mass of nervous tissue. The eye-muscle nerves can be seen in the diagram (fig. 5). From the muscles arise nerve bundles, which run backward for various distances, and end somewhere in the connective tissue, without any connection with the central nervous system. The nerve bundle coming out of the easily

recognizable trochlear muscle, ends abruptly beside the gasserian ganglion, whereas the nerve from the superior rectus runs far backward and can be traced behind the thalamus to very near the dorsal median line, where it comes quite close to the same nerve from the opposite side, without, however, crossing as the trochlear nerve does. This must be the oculomotor nerve, which in the embryo originates far backward from the mesencephalic flexure. From the external rectus muscle nerve fibers can be traced to the trigeminal nerve, perhaps consisting only of sensory fibers from the muscle spindles. I did not follow the other branches of the oculomotor nerve, but the details given in the diagram are essentially the same on both sides.

The trigeminal nerve is represented by a large gasserian ganglion, from which branches divide into their various peripheral end organs. From the ganglion a huge root runs backward, passes the petrosal bone, and spreads out in the loose mesenchymatous tissue; here it ends in connection with the horny covering of the skin defect, or just underneath this (fig. 7). I have not been able to find a motor branch of the fifth nerve, so this must be absent at least in the intracranial part of the nerve.

The branches of the facial nerve form a distinct bundle of fibers, running into the petrosal bone, where the geniculate ganglion can easily be discovered. From this ganglion we can trace the chorda tympani fibers, joining the trigeminal nerve. The larger part of the facial nerve fibers, however, pass this ganglion without any connection, and run in a central direction for some length alongside the eighth nerve, which has gathered its fibers from the cochlear and vestibular organs, where their respective ganglion cells can be easily recognized. These fibers can be traced for a short distance inside the skull, where they end abruptly without any connection with the central nervous system, although quite close to it, and separated from it only by the meninges.

The glossopharyngeal and vagus nerves cannot be distinguished from one another. They begin as a very shallow

bundle of fibers just inside of the foramen jugulare, and form a ganglion jugulare. They then run outward and downward to form a large ganglion nodosum. Many small branches go off here, partly to the upper cervical ganglion of the sym-

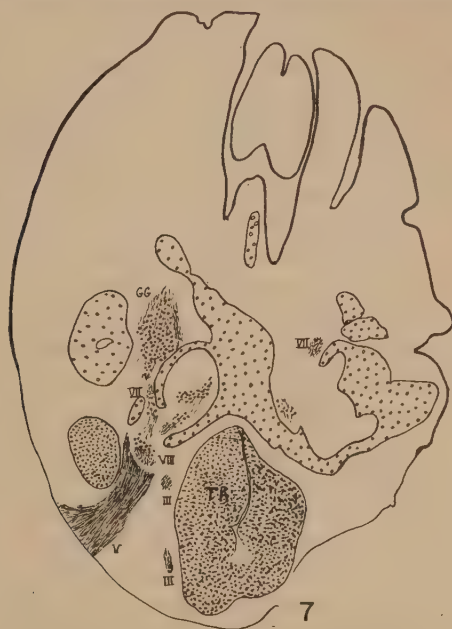


Fig. 7 Drawing (section 63), showing cranial nerves. *III*, The branch of the oculomotor nerve, cut twice, going posteriorly; *GG*, the gasserian ganglion; *V*, root of trigeminus, ending in the thin horny layer; *VII*, facial nerve, lying between the gasserian ganglion and the acoustic nerve (*VIII*). The shaded fields are central nervous tissue; to the left the tip of the temporal lobe; in the center the thalamus (*th*). The mouth is open; the tongue lies inside, as a separate mass between the alveolar process.

pathetic nerve. The main nerve trunk goes downward and can be followed to the stomach; a recurrent laryngeal nerve is quite clearly seen. This nerve is supposed to carry non-sympathetic motor fibers, but normally it probably carries also a number of sensory fibers from muscle spindles. The accessory nerve is represented, as the diagram shows, only

by a small nerve bundle running from the meninges to the sympathetic ganglion. Motor fibers running to the sternomastoid or trapezius muscles cannot be found.

The nerve trunk of the hypoglossus can be followed from the tongue muscles backward as a very small bundle, not half as thick as the vagus. It crosses the ganglion nodosum, with which it has probably connections; its stem is then very much thinner and continues centrally to the first cervical ganglion. It is clear, therefore, that a true hypoglossal nerve is not present, but we find that some fibers from the first cervical ganglion and others from the vagus ganglion run in the twelfth nerve course, perhaps together with peripheral parts of the true hypoglossus, in the same manner as we found for the facial and oculomotor nerves.

The cervicothoracic sympathetic trunk is, as has already been pointed out, quite large, and seems normal in its distribution, its ganglia, and connections. That the ganglia get branches from the spinal ganglia has also been mentioned above.

Histological examination of the nerves, although difficult because of the maceration of the tissues, gives the impression that there is no difference in structure between the most peripheral parts of the eye-muscle nerves and the facial on one side and the vagus and spinal nerves on the other; but the nearer we approach the central ending of the first-mentioned nerves, the more the histological picture becomes indistinct, so that in the central part only a loose meshwork is present, probably consisting only of the cells of Schwann's sheath. This makes it probable that these nerves are actually degenerating, but their most peripheral parts are still normal.

SUMMARY

1. The ovum described above is approximately three months old; the embryo within has followed a slower course of development, so that it is, generally speaking, comparable with an eight weeks' human embryo. The hands and the cerebral hemispheres are a little (perhaps one to two weeks) in advance of other organs. (See also 6, *infra*.)

2. Representing the central nervous system, the hemispheres and the thalamus are present, and the former are normally developed. The hemispheres show folding of the thinner parts of the wall, as often noted when the state of preservation of embryos is not perfect. The thalamus shows extensions to both sides, of unexplained significance.

3. The mesencephalon and hindbrain are absent. As the motor nerves from these parts are developed, the motor ganglion cells must have been present in their neuroblastic stage or later, and they must have disappeared afterward. Whether they were part of a, theretofore, normal neural tube, or merely isolated groups of neuroblasts with retained power of development, cannot be made out.

4. Of the cervical and thoracic parts of the spinal cord no trace can be found. No motor roots are present. Of the lumbosacral part of the cord some remains are still present in a normal meningeal sac. As there are no anterior roots present in this part, we may conclude that a normal cord has not existed as such, but that some parts of the anlage of the spinal cord have differentiated into the marginal zone and cellular zone that we found. The normal closing of the mesenchyma around the spinal cord is in favor of a late destruction. Originally, a spinal cord must have protruded in the opening of the skin of the back, as we find also in cases of pure anencephaly, where the cervical part of the cord forms the center of the area cerebrovasculosa of the brain.

5. The horseshoe-shaped layer of cubic epithelial cells, bordering the skin in the anterior and lateral parts of the area cerebrovasculosa, is not yet fully explained. This can be a persistent, non-differentiated part of the neural plate.

6. Mitotic figures in the brain and other tissues point to death of the embryo shortly before abortion took place.

7. The abnormally straight back and the absence of the normal neck flexure are easily explained as secondary to a faulty development of the central nervous system. This latter, by growing faster in normal embryos than the noto-

chord and primitive vertebral column, gives rise to the typical flexures of the back and head.

The literature does not supply many cases of anencephaly or amelia in very young embryos. I was able to find the records of only five human cases, of which I will give a brief abstract.

Lebedeff, as early as 1881, was the first to describe an early case of the deformity that interests us now. The length of the embryo is 9 mm. The amniotic sac has reached the size of an egg and the umbilical cord is 4 mm. long. The normal flexures of the back and neck are absent; the embryo is straightened, with slight lordosis of the thoracic part. The forebrain vesicles are formed, but their walls are folded, so that the vesicles do not protrude over the eyes, which seem to be normal. The thalamus opens dorsally; its ependyma is continuous with the skin. From that point caudally the medullary groove is open, but although Lebedeff does not definitely say so in his very short description, I infer that it closes again at the lumbosacral level, and that the lower part is covered by skin. Part of the exposed medullary plate is degenerated, and in these places the mesodermal tissue lies open on the surface, but small particles of nervous tissue have grown into this tissue and are still persistent. No mention is made as to the cranial nerves, auditory vesicle, or spinal ganglia.

Frazer described, in 1921, a human embryo of 17 mm. with anencephaly, in which the oblongata was still present (so-called hemicephaly). The general bodily development points to a nearly eight-weeks-old embryo. Eyes and ears are present, as is the spinal cord. Of the brain only the oblongata and the optic stalks are present. The pituitary gland could easily be found; some very irregular nervous tissue lies around it, and it looks as if the optic nerves end here. Portions of all cranial nerves are present; the oculomotor seemed degenerated, the others rather normal. None of these nerves, however, runs into the cranial cavity; they end some-

where outside or in the meninges. Even the last pairs are not present between the base of the skull and the oblongata (Frazer does not give any data about nerve roots or their nuclei in the oblongata). According to Frazer, the nerves must have been broken off by a straightening process of the embryonal flexures of the brain, but he does not say at which period this should have occurred. As he gives very few further data, it is not possible to form an opinion about the process of destruction in this case. Frazer himself thinks that the brain was not normal when the catastrophe occurred.

An embryo described by Cull of nearly the same length as Frazer's embryo, was in such a bad state of preservation that no details could be made out. There was rachischisis, with some nervous remains between the spinal ganglia, covered by loose cellular tissue. Hemispheres and brain stem were present, covered by the same tissue. An opening in the vertex led into the cranial cavity.

The fourth case is that of Böhmig—an embryo of 20 mm., very much like the monster described by me above. As, however, Böhmig's interest lay chiefly in the skull, his description of the nervous system is rather scanty. The age, according to the length of the embryo, is six weeks; according to the structure, however, it is probably one to two weeks older. No data about the membranes were available. The back is straight and the head slightly bent upward. Remnants of the brain are covered by a membrane with an opening in the center. Hemispheres could be made out, but the poor preservation of the embryo made it impossible to form an exact opinion about the thalamus. The mid-brain and hind-brain were absent. The cervicothoracic part of the back was covered by skin, underneath which the spinal ganglia were lying just in the same way as in my specimen. No meningeal cavity was present. At the level of the seventh thoracic vertebra the skin opened; the spinal cord appeared in the opening and seemed in direct continuation with the skin. From there downward a meningeal sac existed, covered by normal skin, and containing spinal cord. It was impossible

to make out whether this had been normal up to the death of the embryo and macerated afterward or was already abnormal before death. No reference is made to anterior nerve roots.

A fifth case, somewhat similar, is given by Mall. The human embryo was 20 mm.; the amnion sac, 80 mm. There had been endometritis; the chorion contained many purulent foci. No skin was present over the brain, an opening in the mesodermal membrane at the vertex exposed the brain, but did not lead into the ventricle. The eyes had grown together; there was one optic nerve. The brain ended posteriorly in a mass of tissue back of the ears, of which no further description is given. The back is covered with smooth skin. The spinal cord is present from the upper cervical to the upper lumbar region and seems to be normal in this part; caudally, it ends in a fibrous mass. Downward from this point the spinal canal (i.e., vertebral canal) is filled with mesodermal tissue. Spinal nerves and ganglia are present in this latter part.

I will not try to give a review of the literature on rachischisis, because our knowledge of the various forms of this deformity is not yet sufficient to allow us to give a general explanation of this anomaly, either as to cause or as to morphogenesis.

There are still some obscure points, which have to be cleared up by detailed study of the specimen, before we can venture upon a general hypothesis. I will mention three questions which presented themselves to me during the study of the embryo. First, what is the significance of the layer of cubic epithelium which lines the anterior end of the area cerebrovasculosa, as described above? This is so typical a structure, that its presence cannot be merely accidental, but must have some bearing on the ulterior development of the tissues which lie in the very young embryo in the frontal part of the neural plate.

A second point which can only be solved by the study of adequate material is the significance of the opening in the

vertex of the head. This opening was found in four out of the five cases of anencephaly in young embryos referred to above, whereas in the fifth case (Frazer's case) the injury to the skull makes it impossible to know whether such an opening had been present or not. Of fourteen cases of anencephaly in the collection of the Department of Anatomy of the Peking Union Medical College, which I could examine in regard to this point, I found the opening present in eleven, doubtful in two, and absent in one. This last case had no remnants of brain whatever (a rare finding), and the normal skin of the face did not come up to the level of the eyebrows in the median line, but ended on the back of the nose. In several other cases in literature this opening has been described, but explained as traumatic. Lebedeff remarks that it leads into the brain cavity in his case and is lined with ependyma, which is continuous with the skin. I was able to examine a case of hemicephal (partial anencephaly) in serial sections, and there too the opening was present, but led into the rhombencephalic ventricle. This opening cannot be the anterior neuroporus, as that structure lies at the frontal end of the neural tube, somewhere in the lamina terminalis, near the preoptic recess.

In discussing the morphogenesis of the above-described monster, I think we are safe in saying that the *injurious agent or trauma operated after the outgrowth of the motor roots of the cranial nerves from the central nervous system*. This process begins in embryos of about 10 somites, 2.1 to 2.5 mm. in length. There is a rather considerable variation in the various early human embryos reported, but in this stage the neural tube has closed in its cervical part. According to some authors, closing of the tube begins in the mesencephalic region; according to others, in the upper cervical region, and proceeds from there in frontal and caudal directions. It may be that the motor roots develop before the closing of the midbrain or after it, but the upper part of the spinal cord is always closed at that period. The embryo is still straight, only the prosencephalic groove is bent at about

a right angle to the axis of the body, the diencephalon forming the top of the flexure. At the caudal end the neural groove is practically straight, but the primitive streak is also bent ventrally. There is no kyphosis. In some embryos there may be even a slightly lordotic curvature. Bartelmez and Evans state that most of the bends and flexures found in young human embryos are postmortem changes, due to collapse and wrinkling of the yolk sac.

The maximum lesion lies in most cases of rachischisis in the mesencephalic region. In the ordinary cases of anencephaly, as we know them from the text-books of pathology, nothing is left of the mesencephalic structures except the motor nerve fibers in the periphery and the gasserian ganglion with its branches; all central nervous tissue has disappeared. The diencephalon is rarely totally destroyed, the chiasma and infundibular region being present in many cases, often with more or less nervous tissue around them. In the above-described case, as in those of Böhmig and Lebedeff, the thalamus was present. Remains of the forebrain are always present; olfactory lobes could be found in nearly every case, and structures resembling the choroid plexus are also a very common finding. The choroid plexus develops as a fold from the mesial wall of the hemisphere, and is therefore a differentiation from the dorsal part of the neural tube. A great many anencephalics have in the area cerebrovasculosa more or less differentiated islands of nervous tissue, in which Veraguth was able to find ganglion cells arranged more or less as a brain cortex. These also must therefore belong to the dorsal structures.

At the occipital end we often find a total absence of the hindbrain, as in all cases in which anencephaly is combined with amyelia; but we know, too, that when the spinal cord is not involved, more or less tissue of the rhombencephalon may still be present; in some cases (called hemicephaly or partial anencephaly) even the entire oblongata and the basal part of the pons are formed (de Vries). From this we may draw another conclusion: *The dorsal parts of the neural axis are always affected more than the ventral parts.*

Looking to the spinal cord, we find facts in favor of our first conclusion. Speaking about the vertebral column in rachischisis, Kermauner says that in all cases within his knowledge the sacral bone was closed. When we examine the remnants of spinal cord in these cases, we often find that in the cervicodorsal part no nervous tissue is left, whereas in the lower part the cord lies flattened out as area medullo-vasculosa, often with ganglion cells still present, from which anterior roots originate. In some specimens of the collection of the Department of Anatomy (Peking Union Medical College) I found the upper part of the cord present as a plate, but the lower part as a solid tube, covered by a thin, transparent membrane. The opposite was never noticed (when we do not take spina bifida into account here), only in one of these cases the upper and lower parts of the cord were both present as plates, whereas the central part was absent. This corresponded in position to the very marked kyphosis of the vertebral column.

In this respect Holmdahl's work is of special interest, in which he states that there is a difference in the first development of the neural tube in the cervicothoracic level from the mode in which it develops in the lumbosacral part of the embryo, where no neural groove exists, but the cord develops at once from a group of indifferent cells. Disunion of this problem and its bearing on the localization of spina bifida would lead us too far in the present discussion.

This conclusion is in contrast with the fact that spina bifida is found much more frequently in the lumbar part than in the cervicothoracic, so that for its explanation we must probably search in different directions.

The subject of spina bifida brings us to a short discussion of the relation between nervous and mesoblastic tissue in these deformities. There is a general parallelism in the development of the neural axis and its surrounding tissues, but when one is more fully developed than the other, *it is the nervous tissue that is in the better condition*. Strange as this may seem, we shall have to reckon with the facts before

a final explanation can be given. In *spina bifida occulta* great defects of the vertebral arches and dura may exist without any change in the spinal cord. In *spina bifida aperta*, also, skin (consisting only of epidermis, while the corium is absent), bone, and membrane changes are often present to a large extent, while the spinal cord seems normal or simply hydromyelic. The opposite, absence or gross deformity of the spinal cord without any fault in development of the vertebrae or the skin, does not occur. In so-called *amylia* I saw in two cases out of seven the lower part of the cord lying normally (to the naked eye) under a transparent membrane. I could not exactly determine which segments of the cord were present (probably they were from the fifth lumbar downward) and whether they corresponded to the skin covering the sacral region. Kermauner, in his statement that the sacral bone is nearly always closed, does not mention the nervous tissue in this relationship.

In cases of total *rachischisis*, with flattening of the medulla, there is always a very marked lack of connective tissue beneath and lateral to the nervous tissue and an absence of the corium. The pia may be present with its vessels, but the cord is not attached to the vertebrae; the roots run through a cavity, and there is often no trace of bony arches found; but the muscles of the spine are all present, although they cannot take their normal positions.

In *anencephaly* there is, as far as I know, always close correspondence between the development of skin and bone and that of nervous tissue.

The explanation of the spinal-cord findings in my case is difficult; but in view of what has been said about the connective tissue, I am inclined to think that in the lumbosacral part a spinal cord must have been present, which has degenerated afterward from some unknown cause. The remnants of cord found lying in the meningeal sac point to the same conclusion. As far as I know, a similar finding has never been mentioned in literature. The embryo that most closely resembles mine, that of Böhmg, had a normally developed lower spinal cord.

I am not able to explain the findings in the cervicodorsal part, where normal skin covers only the spinal ganglia and the bodies of the vertebrae.

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NOTES ON THE GOLGI APPARATUS IN SPINAL-GANGLION CELLS OF THE CAT

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THREE FIGURES

This work was undertaken with the primary purpose of studying the differences in impregnation reaction of the Golgi apparatus in the spinal-ganglion cells of cats of different ages. It is well known that the Golgi bodies in young animals are generally more easily impregnated with silver nitrate than in older ones, especially so in nerve cells. Whether this difference applies also to methods involving impregnation with osmic acid has not been definitely established, and the present paper deals particularly with this point. The procedure in this experiment was to impregnate with osmic acid spinal ganglia from cats of different ages (ranging from twelve hours after birth to maturity), the conditions being controlled so that the technique used was reasonably constant throughout.

The method followed was the modification of the Kolatchev technique proposed by Nassonov ('24)¹ for the impregnation of mammalian tissue. It consists, in brief, of fixing the ganglia, entire, in Champy's fluid (2 parts of 3 per cent bichromate of potassium, 2 parts of 1 per cent chromic acid, and 1 part of 2 per cent osmic acid) for twenty-four hours; washing in running tap-water for twenty-four hours; osmicating in 2 per cent osmic acid for from three to eight days, the first eight hours of osmication being carried on at 40°C., the remainder at 35°. After osmication, the tissue is again

¹ Nassonov, D. 1924. Das Golgische Binnennetz und seine Beziehungen zu der Sekretion. Arch. f. mikr. Anat. und Entwickl., Bd. 100.

washed in running tap-water for twenty-four hours and dehydrated and embedded as usual. The sections were cut 5μ thick. Ganglia from all portions of the nerve cord were obtained, and those from the different regions were kept separate. The dissection was made in Ringer's fluid, and in general all of the tissue was in fixative not much later than half an hour after the death of the animal, most of it much before that time.

The results obtained agree, in the main, with those which have been observed after silver impregnation. The most successful impregnations were obtained in the youngest animals (about twelve and sixteen hours after birth) after two and one-half to three and one-half days of osmication. This left the Golgi material blackened as usual, with, however, certain differences in apparent intensity throughout the strand—a matter which will be discussed presently. The nucleus was left unstained, with the exception of the nucleoli and occasionally a few strands of what is probably linin, which turned yellow, as did the cytoplasm proper. An illustration of a typical impregnation such as this is given by figure 1, a spinal-ganglion cell of a sixteen-hour kitten after three and one-half days' osmication. If osmication is allowed to go on for a longer period, the Golgi material becomes progressively overimpregnated, as shown by excessive blackness and coarseness of the strands as well as darkening of the cytoplasm. It is of interest to note that in another animal of the same age, where 1 per cent osmic acid was used for osmication, about seven days were required for a comparable result, or twice the normal time for proper impregnation of the Golgi apparatus with some blackening of the background. It is highly improbable, however, that such a quantitative relationship as this would hold throughout, especially since prolonged osmication owing to low-strength reagent would result in excessive blackening of the cytoplasm.

In older kittens, i.e., about seven days after birth, the time for best impregnation by the usual method was found to be considerably increased, being from five and one-half to

six and one-half days, or approximately twice the time required for twelve-hour kittens. Nor were the results at their best quite so good as in the younger ones.

In the adult cat it was found impossible to obtain impregnations comparable in general excellence with those of younger animals. At best, after about six or seven days, the strands are faint and frequently not much different from the surrounding cytoplasm, which seems to have blackened somewhat more readily than in the younger animals. Figure 3



Fig. 1 Spinal-ganglion cell from a sixteen-hour kitten after three and one-half days' osmication with 2 per cent osmic acid.

shows a ganglion cell from an adult cat after seven days of impregnation. The faintness of the strands and the blackening of the cytoplasm is typical. The shrinkage of the cytoplasm from the capsule is probably due to improper fixation and occurs only occasionally.

In no case was there any appreciable difference between impregnations of ganglion cells from different regions of the cord in the same animal.

While studying the preparations, two matters of interest were noted. In the more delicate impregnations the Golgi material appears not in the form of fine threads or canals,

but very frequently as plate-like or flat ribbon-like structures of dark grayish substance. Where intense blackening appears, it is usually at points along the edges of these plates. This is well illustrated by figure 2, from a twelve-hour kitten, and to some extent by figure 1 from a sixteen-hour kitten. The probable explanation of this is that the Golgi material is in the form of a plate-work with certain portions along the edges turned up or down at right angles to the plane of the slide, being seen, therefore, in optical section and giving

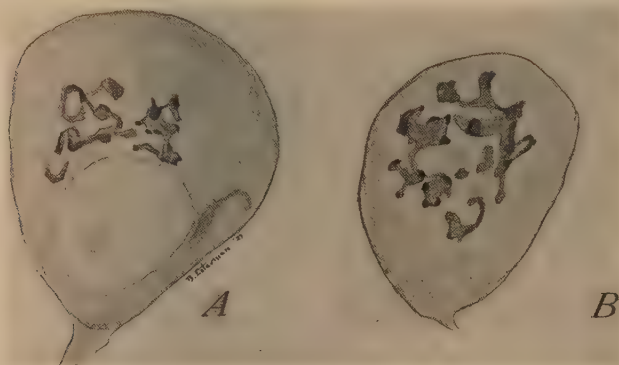


Fig. 2 (A and B) Spinal-ganglion cells from a twelve-hour kitten after seven days' impregnation with 1 per cent osmic acid, showing 'platework' of Golgi apparatus.

the plate the deeply blackened appearance in spots along the edges. If the fact that the Golgi material in all of these preparations is consistently in the form of more or less discrete bodies, even though it frequently appears like a connected network on superficial examination, were not sufficient to show the improbability of a system of canals, as suggested by Holmgren, certainly the flattened appearance of these strands would be conclusive evidence against this view.

It was found impossible to duplicate, by using the Kolatchev method, any of the continuous networks of fine, anastomosing threads, such as were originally figured by Golgi ('99)² and

² Golgi, C. 1899. Di nuovo sulla struttura della cellule nervose dei ganglii spinali. Boll. Soc. Med.-Chir. di Pavia, 1899.

others who used silver-nitrate impregnations on the same type of tissue. It is now generally conceded, however, that the osmic-acid methods give a far more delicate and accurate impregnation of the Golgi apparatus than do the silver methods.

The second matter of interest was the demonstration of the Golgi apparatus in the cells of the capsule surrounding the ganglion cells. The apparatus is perfectly constant and appears in all the capsule cells when the tissue has been

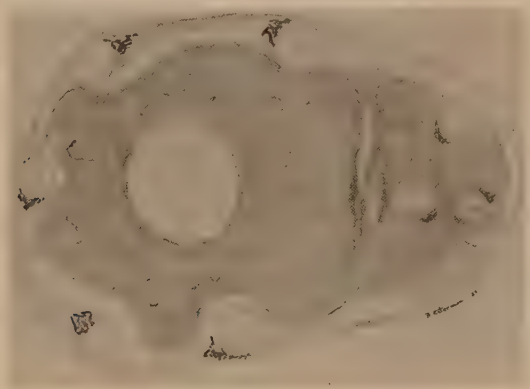


Fig. 3 Spinal-ganglion cell from an adult cat after seven days' osmication with 2 per cent osmic acid, showing the Golgi apparatus in the cells of the capsule.

sufficiently intensely impregnated. The conditions are well shown in figure 3, which is from an adult cat after seven days of osmication. The fact that the cell itself has undergone some shrinkage makes this a favorable demonstration, since it leaves the capsule proper more distinct. As shown here, the Golgi apparatus appears always localized at one side of the nucleus in the form of a black, tangled mass. Their extremely small size prevents a more detailed study of the morphology of the strands. The presence of the Golgi material in the capsule cells has rarely been described, and its occurrence in these cells, whose probable function is merely to

protect and insulate, only goes further to indicate its universal importance in generalized cell processes.

The results of these experiments indicate clearly that, as with silver nitrate, so also with osmic acid, the Golgi material reacts more readily in the nerve cells of young animals than in those of old ones. This suggests the possibility that similar conditions may exist with respect to other classes of tissue, and suggests that particularly refractory material might be most advantageously studied in young rather than adult animals. The fact that some change goes on in the Golgi material as the animal is attaining maturity immediately suggests the possibility that this substance is in some way connected with development and differentiation, in this case, of the nervous system. It is interesting to note in this connection that the greatest change takes place during the first week or so in the life of the animal. It is not so difficult to draw a parallel between differentiation of tissues and completely intracellular differentiation of single cells. And the importance of the Golgi substance in intracellular differentiation as shown by the formation of the acromosome in spermatozoa has been conclusively demonstrated by Bowen and many other workers following him.

We should like to express here our indebtedness to Prof. Robert H. Bowen who gave us freely of his experience in cytological technique and whose advice and guidance we have greatly appreciated.

OBSERVATIONS ON THE LYMPHATIC CONNECTIONS OF THE THYROID GLAND¹

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FOUR FIGURES

INTRODUCTION

The lymphatic connections of the thyroid gland are of interest because of the differences of opinion that exist concerning the paths by which the secretion reaches the blood. The lymphatic channels are of importance in functional, inflammatory, or malignant disorders, because of their close relationship to adjacent structures, chiefly the thymus.

The observations recorded here are the results of studies of the lymphatic vessels of the thyroid gland and their relation and connection with adjacent structures. Dogs, swine, rabbits, guinea-pigs, two calves, and a colt were used in the experiments.

LITERATURE

In the first volume of Guy's Hospital Report, King noted that the thyroid lymphatics in man were "of large size and great number and their absorbent vessels carry its peculiar secretion to the great veins of the body." In this same volume is a series of communications by Sir Astley Cooper concerning the thyroid gland, its chemical composition, and

¹ Submitted for publication July 23, 1927.

some of its vascular and lymphatic connections. Since this time, many studies have been carried out concerning the lymphatic vessels of this gland and their place in the physiology of the organ.

Carlson and Woelfel demonstrated considerable variation in the flow of lymph from the thyroid gland of dogs. The extreme range of flow was from about 2 cc. in twenty-four hours to 60 cc. in twenty minutes. The latter was seen in a dog with carcinoma of the thyroid. As far as these observers could determine, the only differences between the thyroid lymph and ordinary lymph in the neck was that the latter contained more lymphocytes. In dogs weighing 12 kg. with large goiters Carlson and Woelfel found that more than 200 cc. of thyroid lymph was emptied into the circulation within twenty-four hours.

Williamson, in 1926, described a 'leash' of lymphatic vessels in cadavers extending from the thyroid gland along the trachea to the thymus. Cooper demonstrated that the absorbent vessels in the stalk were connected with the thyroid.

Hicks, duplicating studies of Carlson, Hektoen, and Schulkof, and of Hektoen and Schulkof—found that thyroglobulin was present in both thyroid lymph and venous blood from the thyroid gland. It was not demonstrated in the arterial blood. It must be remembered, however, that thyroglobulin cannot be considered as an index of the amount of thyroxin output of the thyroid.

Mann has demonstrated that iodine (Lugol's solution) causes an increased flow of lymph from the head region of dogs. Our own observations concern chiefly the lymphatic connections of the thyroid gland with especial reference to the thymus, adjacent lymph nodes, and veins.

EXPERIMENTS

Animals operated on were anesthetized with ether and placed in a dorsal recumbent position, and the thyroid gland approached through a median-line incision. It was important that the incision be made as near the median line as possible.

The dissection was carried down through the intermuscular fascia to the trachea. Hemorrhage was avoided as much as possible, since blood in the connective tissue obscured the operative field. The thyroid lobes were carefully exposed by blunt dissection, elevated into the incision, and held with a gauze sponge until injected. Freshly diluted ink (one part Higgins' American India ink to five parts of sterile distilled water) was injected with a glass-plunger syringe through a 23- or 25-gauge hypodermic needle just beneath the capsule of the gland. When the injection was properly made, almost the entire gland became black at once and ink was visible in all of the lymphatics leading from the thyroid. The quantity of ink injected depended on the size of the gland; in the average normal dog about 0.5 to 1 cc. was sufficient. Care was always exercised not to drop ink on the outside of the gland or in the operative wound during or after the injection. We have injected ink solution into the thyroid capsule within two hours after death while the body was still warm and obtained good flow through the lymphatics. Massaging increased the flow of lymph and aided in making the ink go to more distant points.

Twenty to thirty minutes after the injection, the animals were killed under ether and the neck and thorax carefully dissected. Many observations demonstrated that this time interval was necessary in order to obtain complete injection. After a longer interval, the ink may leave some of the larger vessels.

RESULTS

The lymph drainage of the thyroid gland was variable. In the dogs the systems of drainage fell into three groups:

Group 1. From the anterior pole of each lobe the lymph passed to a lymph node at the angle of the jaw and then into the cervical lymphatic trunk. From the posterior pole of the thyroid gland lymphatic vessels passed to a series of lymph nodes extending along the ventral surface of the trachea and on into the thymus and mediastinum (fig. 1). In this group the ink was usually visible in the thymus tissue on histologic section.

Group 2. The drainage from the anterior pole was the same as that in group 1, but the lymphatic vessels from the posterior pole of the thyroid emptied directly into the cervical

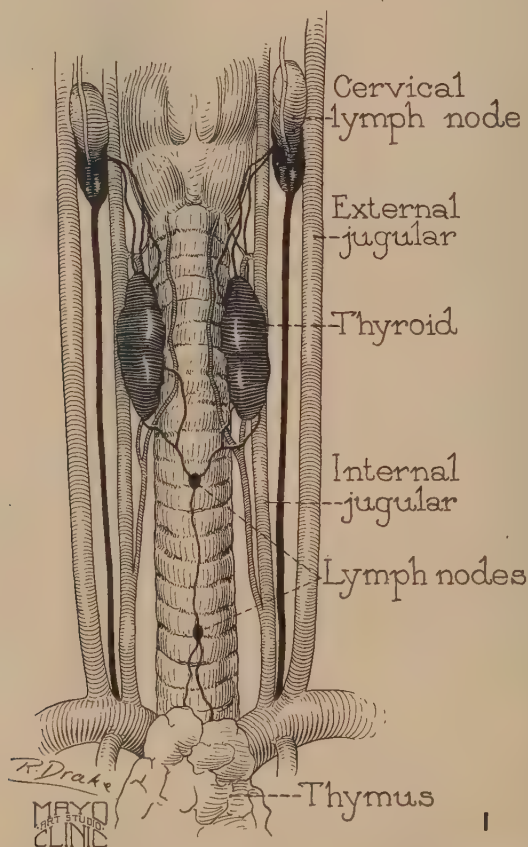


Fig. 1 Lymphatics of the thyroid gland of a dog emptying into adjacent lymph nodes and thymus.

lymphatic trunk and thence into the venous circulation at the point of juncture of the external and internal jugular veins. In these animals the lymphatic vessels coming from the posterior poles of the thyroid did not pass through any

lymph nodes before entering the general circulatory system (fig. 2). Ink was not revealed in these cases on careful histologic examination of the thymus.

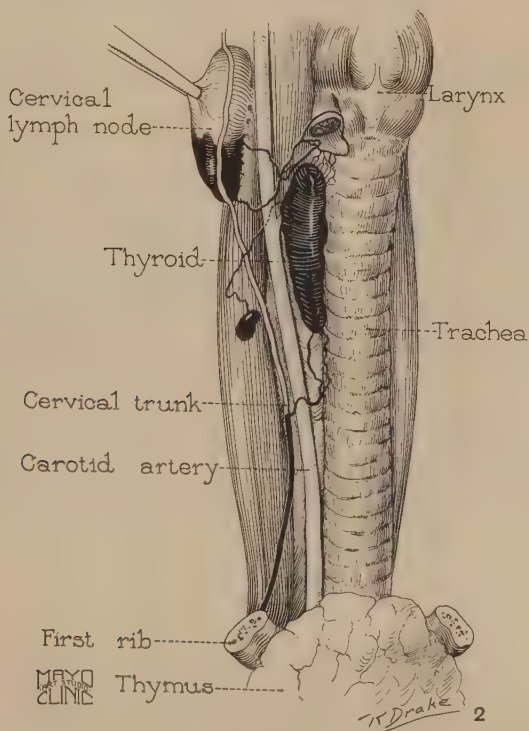


Fig. 2 Lymphatics of the thyroid of a dog emptying into the cervical node and cervical lymph trunk.

Group 3. The drainage was the same as that in group 2, except that the lymphatic vessels coming from the posterior pole of the gland emptied directly into the internal jugular veins (fig. 3), and, as in group 2, ink was not found in the thymus.

In groups 2 and 3 ink was recovered from the blood at the point of entry of the lymphatic vessels. Sections were cut from the vein and lymphatic vessels at their point of confluence and prepared for microscopic study (fig. 4).

In swine the thyroid lymph drainage was much the same as that noted in the dogs of group 1. There was usually more ink in the thymus, but this may have been due to the fact

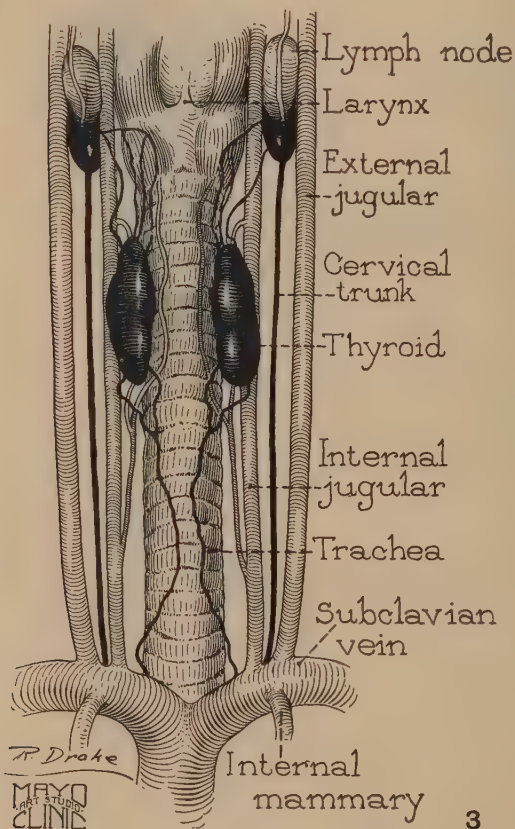


Fig. 3 Lymphatic vessels from posterior pole of thyroid gland emptying directly into a vein.

that the thymus lies immediately adjacent to the thyroid gland.

In the colt and calves there was abundant lymph drainage, but in none of these animals did the lymphatic vessels empty

directly from the thyroid gland into the venous circulation. The lymphatic vessels arising from the anterior pole of the thyroid gland all terminated in an anterior cervical lymph node, while those arising from the posterior pole terminated in the other cervical lymph nodes and in a number of small nodes on the ventral surface of the trachea extending to the mediastinum.



Fig. 4 Lymph channel, *a*, entering the vein; *b*, valve at the juncture of the lymph vessels and vein.

In all of the animals observed the lymph which drained from the thyroid to the anterior cervical lymph node drained only into the posterior third of this node and from there through the cervical trunk to the venous circulation.

In order to be certain that we were dealing with lymphatic vessels and not veins, especially in the animals in which direct connection was seen between the lymphatic vessels and veins, we studied the structure and contents of the vessels. It was noted that the lymphatic vessels were almost invisible channels filled with clear lymph, and after the injection of the ink solution the vessels turned black.

COMMENT

In our earlier experiments trypan blue was employed as the injection medium, but it was not entirely suitable, for it rapidly permeated the walls of the vessels into the surrounding structures. A clear-cut injection could be maintained for only a short period. Higgins' India ink in a dilution of 1:5 was found to be a satisfactory medium for injection. This dilution was enough to fill the lymph channels rapidly without permeating the tissues to any great extent, and yet it was maintained for some time after injection in the lymphatics; that is, the lymphatic vessels and nodes were visible for several hours or even days. An objectionable feature of India ink is that if one wishes subsequently to section any of the tissues from within the thorax for histologic study the ink is indistinguishable from the ordinary carbon pigment seen in anthracosis. We overcame this objection by using country-bred or young dogs that were free from anthracosis.

In approximately two-thirds of the dogs examined (groups 2 and 3) it was noted that the lymph drained from the posterior poles of the thyroid gland into either the cervical lymphatic trunk and thence into the general circulation, or directly into the veins at the base of the neck. In these groups lymph from the posterior poles of the thyroid did not pass through lymph nodes. This observation may be of importance if it is found that the thyroid secretion passes from the gland through the lymph. In cancer of the thyroid, for example, because of these different lymphatic connections, the disease could readily metastasize to adjacent lymph nodes, the thymus, and through the direct lymphatic vessels into the general circulation and lungs. It may be that these same direct communications of lymphatic vessels with veins obtain in other organs; if so, they are of physiologic and pathologic importance.

Baume has found in cattle that lymphatic vessels may empty into veins or the thoracic duct. His observations were made in cattle, and most of the connections studied were in the abdominal cavity.

SUMMARY

The lymphatic connections of the thyroid gland with regional lymph nodes and venous circulation are varied. In the animals observed India-ink solution injected into the thyroid gland just beneath the capsule was sometimes found in the adjacent lymph nodes, cervical trunk, or thymus. However, in about a third of the dogs examined the lymphatic vessels pass from the posterior poles of the thyroid gland directly into the veins at the base of the neck. Injection may be made into dead animals with good results if the procedure is carried out before the body is cold.

These studies may be of importance in the future consideration of the physiology and pathology of the thyroid gland.

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ROTATING FUNCTIONS OF THE ILIACUS AND PSOAS MAJOR MUSCLES

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FOUR FIGURES

Reference to sixteen anatomical text-books for the rotating functions of the iliacus and psoas major muscles shows that five favored these muscles as medial rotators, six as lateral rotators, and five made no mention of the rotating functions. The work of E. W. Thomas¹ on the rotators of the hip-joint was a most complete study, and showed that these muscles could act as either medial or lateral rotators. However, from the work done in this laboratory, we believe that the lateral rotating function is only secondary, and not a primary function.

Confronted by these extremes of opinion, we made an attempt to determine the rotating functions of the iliacus and psoas major muscles. A mechanical device as shown in figure 1 was constructed. The pelvis with the intact hip-joint and femur was clamped to the table. A cord was attached to the insertion of the muscle and then directed toward the origin of the muscle (fig. 2). The cord, instead of being attached to the origin, was run through a hook screw and over a pulley across the table, where a weight was attached to the end of the cord. The weight kept the cord at constant tension and a needle was so fastened in the cord as to register all movements. When the femur was rotated, the needle indicator held against a chart moved through a distance equal to the elongation of the muscle. Then, on

¹ E. W. Thomas: *The Philadelphia Monthly Journal*, vol. 1, no. 1, 1899.

rotating the femur back to the normal position, the contraction distance was recorded. To get the action of groups of muscle fibers in the various parts of the muscle, the cord was passed through a various number of hook screws set at intervals over the origin of the muscle.

The rotation of the femur takes place on an axis running from the upper medial articular surface of the head of the femur to a midpoint in the intercondyloid fossa (fig. 3). The lesser trochanter (fig. 3) is the functional insertion of both

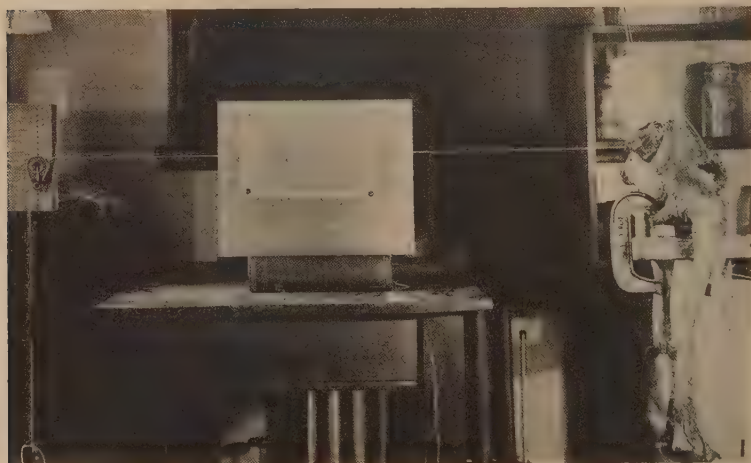


Fig. 1 Apparatus used in experiment. Pulley may be lowered or raised on block (left) to keep cord level. Wire indicator moving over ruled paper registers amount of motion.

the iliacus and psoas major muscles and is always lateral to the axis of rotation whether the femur is extended, flexed, abducted, or adducted. Therefore, any force from an anterior superior medial position, such as indicated by the lines of traction of the iliacus and psoas major muscles, must rotate the femur internally.

The cord was run from the insertion to three different points of origin of the psoas major muscle, and from the chart (fig. 4) it will be seen that in all positions of extension,

flexion, abduction, and adduction, the femur was medially rotated. The iliacus muscle was worked out in a similar manner, the cord running from the insertion to the three different points of origin. From the chart (fig. 4) it will be



Fig. 2 Anterior view, showing cord passing from insertion or lesser trochanter to origin on ilium, where it then passes through hook screw.

seen that in both extension, flexion, abduction, and adduction this muscle is a very weak medial rotator. This is probably due to the direction of the lines of traction of the iliacus which are almost perpendicular from the point of insertion.

In the experiment it was found that either the iliacus or psoas major muscle could assist in lateral rotation if the

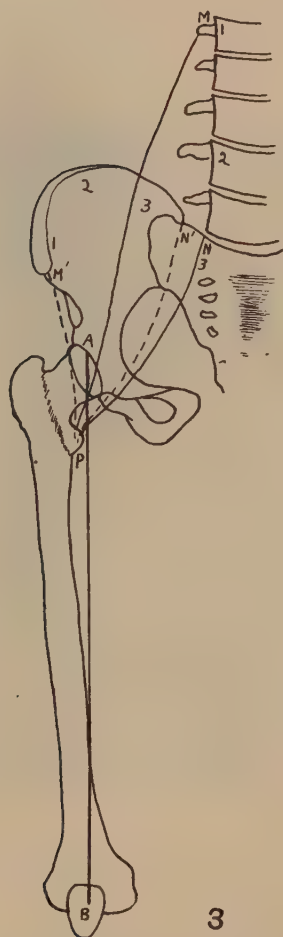


Fig. 3 Line *A-B* is axis of rotation. *P* is insertion of iliopsoas major muscles. *M-P* and *N-P* are lines of traction of psoas major. *M'P* and *N'P* are lines of traction of iliopsoas. The figures 1, 2, 3, are positions of hook screws on muscle origins.

PSOAS MAGNUS

	STRAIGHT			ABDUCTION			ADDUCTION		
	1	2	3	1	2	3	1	2	3
EXTENSION	P								
MEDIAL ROTATION LATERAL ROTATION									
									YES NO
FLEXION	P								
MEDIAL ROTATION LATERAL ROTATION									
									YES NO

ILIACUS

	1	2	3	1	2	3	1	2	3
EXTENSION	P								
MEDIAL ROTATION LATERAL ROTATION									
									SLIGHT NO
FLEXION	P								
MEDIAL ROTATION LATERAL ROTATION									
									SLIGHT NO

4

Fig. 4 Lines extending upward from the horizontal line indicate medial rotation, lines extending downward indicate lateral rotation. P is origin of the muscle and figures 1, 2, 3, are different points of insertion.

femur was first laterally rotated and the lesser trochanter (*P*) was brought over to or beyond the axis of rotation (*A-B*). Although a male pelvis was the nucleus of the work, experiments on the female pelvis and the pelvis of a full-term fetus did not show any alteration in the functions of these muscles except in degree.

CONCLUSION

The psoas major and iliacus muscles are medial rotators of the hip-joint.

OBSERVATIONS ON THE LYMPHATIC CONNECTIONS OF THE THYROID GLAND IN MAN¹

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FIVE FIGURES

INTRODUCTION

In recent experiments on the lymphatic connections of the thyroid gland in dogs it was demonstrated by subcapsular injections of diluted India ink that there are three types of efferent connection, one in the cervical lymph nodes, one in the cervical nodes and the cervical lymphatic trunk, and one directly in the veins at the base of the neck. The present experiments were undertaken to determine if similar variations occurred in man.

METHOD

Twenty thyroid glands in unembalmed bodies were injected with diluted India ink shortly after death. A crescent incision was made below the clavicles, and the skin and subcutaneous tissue was dissected upward above the larynx. The sternohyoid muscles were separated in the median line, cut across, and reflected. The inner borders of the sternothyroid muscles were retracted outward, and approximately 5 cc. of India ink diluted with water was delivered directly into the substance of each lateral lobe, since it was found that subcapsular injection in man was inadequate to secure proper flow of the ink. A glass syringe and a 20-gauge hypodermic

¹ Submitted for publication August 18, 1927.

needle were used. Almost immediately after the injection, the ink was found in the lymph vessels and the regional lymph nodes. The sternothyroid, the sternomastoid, and the anterior belly of the omohyoid muscles were cut and deflected. With some care the ink-containing lymphatics were followed from the gland to their termination.

LYMPHATIC CONNECTIONS

In man the lymph vessels leave the thyroid gland principally at three different areas: the superior pole, the inferior pole, and the middle of each lateral lobe (fig. 1). Lymphatics from the superior pole pass either over or under the thyroid attachment of the sternothyroid muscle. One or both examples may be present at the same pole. These generally follow the course of the superior thyroid artery upward and backward, then turn downward across the carotid artery and the internal jugular vein to enter lymph nodes of the deep cervical group. In three instances we found that the injected material infiltrated a remnant of the thyroglossal duct, and that this structure gave off lymphatics which joined the group from the superior pole. A small lymph vessel was occasionally found in this region even where there was no remnant of the thyroglossal duct. The inferior group of lymphatics originated from the isthmus and the lower poles and ran downward as a leash into the region between the inferior border of the thyroid gland and the left innominate vein, some ending in lymph nodes anterior or lateral to the trachea and some disappearing in the adipose tissue. In only one instance could a lymphatic be traced into the thymus, and then only after it had passed through a lymph node in the region above the left innominate vein. The middle group of lymphatics arises from each lobe and runs directly laterally either anterior or posterior to the carotid artery and the internal jugular vein to enter nodes of the deep cervical group. In one instance a lymph vessel passed between the artery and the vein. However, we were able to demonstrate variations from the usual lymphatic connections. In three different

preparations a lymph. channel arising from the right lobe passed outward anterior to the carotid artery and the internal jugular vein, then turned downward and emptied directly into the right subclavian vein near its juncture with the internal

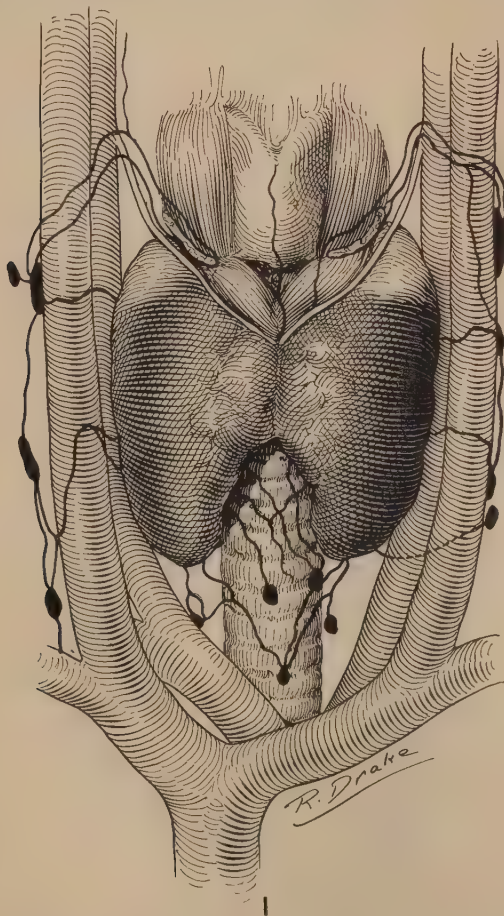
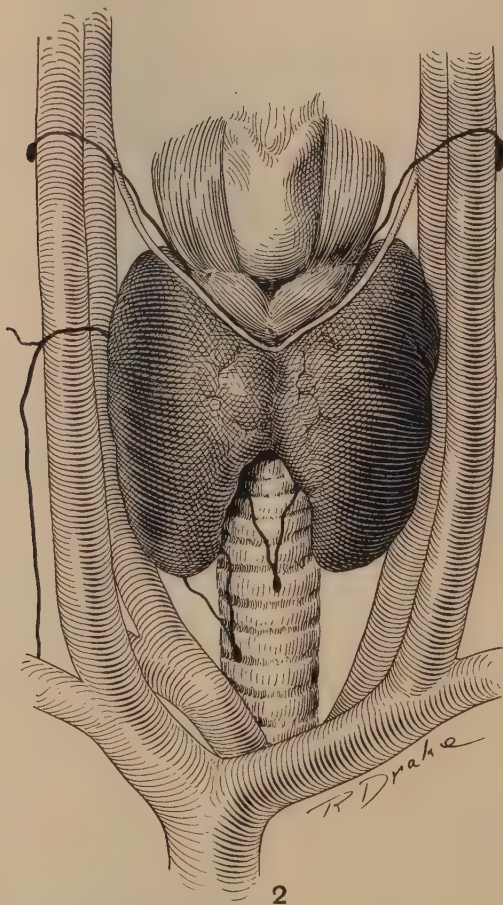


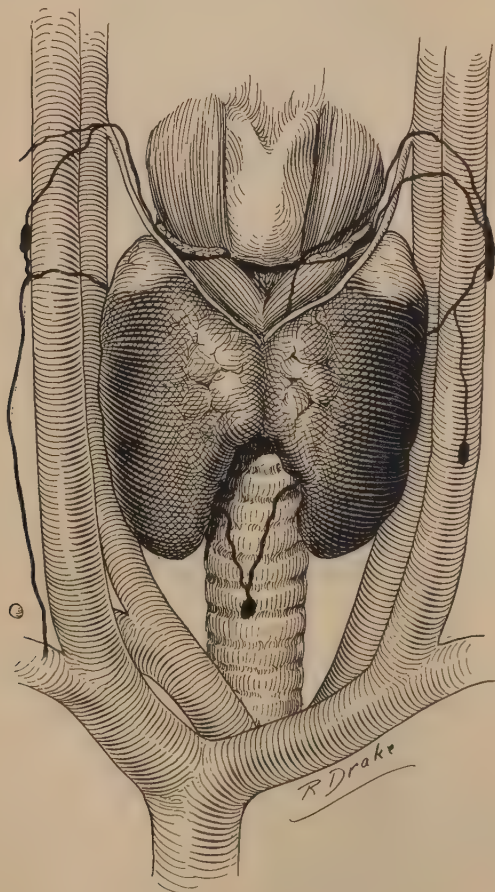
Fig. 1 Composite diagram of the usual lymphatic connections of the thyroid gland.

jugular, without passing through a lymph node (figs. 2, 3, and 4). The lower part of these lymphatics, together with the adjacent tissue and the subclavian vein, were removed and cut serially. Ink was found in the lumen of the lymphatic, which was directly continuous in each instance with the lumen of the vein (fig. 5).



Figs. 2, 3, and 4 Uninterrupted connection between lymph vessel from the thyroid gland and the right subclavian vein.

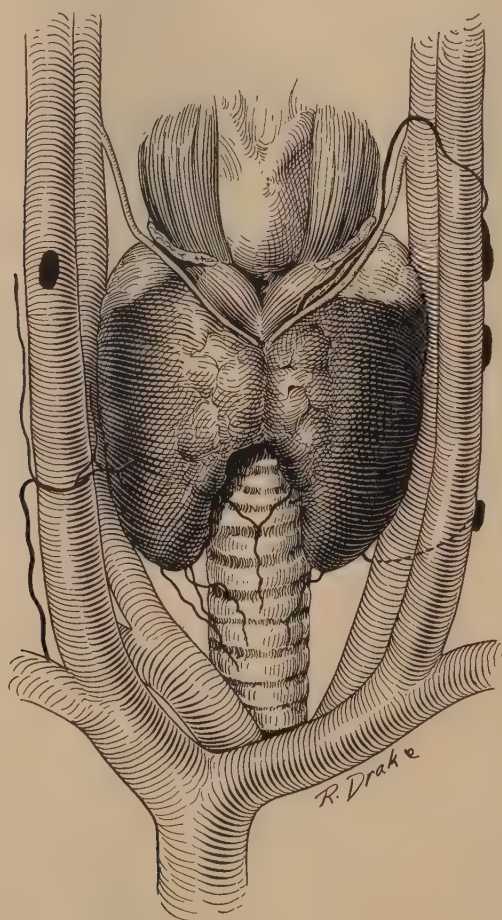
The possibility that the thyroid secretion is lymph-borne has been noted in the literature. Many observers have described a substance in the lymph channels of the thyroid of man similar to the thin secretion found in the acini. In the angler fish (*Lophius piscatorius*) there is a direct connection between the thyroid lymph sac and the heart. The lymphatics



3

Figure 3

in the substance of the gland should be further studied, for there is much discrepancy of opinion as to their distribution. If it is true that the secretion of the thyroid passes out by way of the lymphatics, the demonstration of this direct connection with the vein might be of importance in the physiology of the gland. It certainly is of importance in the metastasis of malignant neoplasms.



4

Figure 4

CONCLUSION

In three of twenty preparations in man there was a direct connection, without the interposition of lymph nodes, between a lymph vessel from the thyroid gland and the right subclavian vein.



Fig. 5 Ink in lumen of lymph vessel which opens directly into the right subclavian vein. Section from serial. $\times 200$.

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QUANTITATIVE CYTOLOGICAL STUDIES ON THE RENAL TUBULES

II. MITOCHONDRIA-CYTOPLASMIC RATIO

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ONE FIGURE

So much has already been written dealing with the cytology of the renal tubules that the advisability of further investigation along this line might appear to be questionable. The new consideration which it is the purpose of these studies to attempt to introduce is that of the quantitative integration of the cellular components which are recognizable microscopically. The nucleus, cytoplasm, mitochondria, and other elements have been repeatedly described by the foremost observers, and we can add but little to the details which they have presented. What seems to us important is to determine as far as may be the relations as to volume and surface which exist between these and other constituents, to ascertain which vary independently and which vary dependently; in other words, slowly to construct a synthetic picture of the kind of protoplasm in the different segments of the tubule, and to relate this by simple experiments with physiological processes. Obviously, success will depend upon the accuracy of the measurements and the number of attributes which it may be possible to determine quantitatively.

In the first paper (Covell, '26) a beginning was made by the determination of the nucleocytoplasmic ratio. Attention was then directed to a quantitative examination of the Golgi apparatus, but this proved so difficult, for technical reasons, that it was decided to make the estimation first in material

better adapted to its study, namely, in nerve cells (Covell, '27). The present contribution relates to the mitochondria and is a continuation of an earlier quantitative study of these structures in the pancreas (du Noüy and Cowdry, '27).

MATERIAL AND METHODS

As in the other studies, albino rats of known age were employed. Thin slices of kidneys removed from twenty-five animals, ranging in age from two days to one year, were fixed in Regaud's fluid. After dehydration and embedding in paraffin, sections were cut 1μ in thickness. They were stained with acid fuchsin and methyl green. Every effort was made to secure a uniform degree of differentiation.

Suitable typical cross-sections of the renal tubules were selected, the transitional regions between the four principal segments being disregarded. The boundaries of the tubule, its lumen, nuclei, and mitochondria were drawn at the table level by camera lucida with an optical combination yielding a magnification of 3050 diameters. The striated border of the proximal convoluted tubule was not considered in the calculations. The surface areas of the tubule, lumen, and nuclei were obtained by means of a planimeter. The total area of the tubule, after subtracting the areas of lumen and nuclei, was divided by the magnification squared (3050^2), and finally multiplied by the thickness of the section (1μ). The resulting value represented the volume of the cytoplasm in terms of cubic microns.

The total mitochondrial surface in the section of tubule was estimated by measuring the total length of the mitochondria. The mean diameter of the mitochondria was obtained by measurements made directly on the tracings. It was found that the mitochondria of the proximal convoluted tubule maintained a diameter of approximately 0.47μ , while the distal convoluted tubule and ascending limb of Henle's loop contained mitochondria with a diameter in the neighborhood of 0.35μ , and that those in the descending limb of Henle's loop were about 0.24μ in diameter. After the total

length and diameter of the mitochondria had been taken, the surface area was then computed as for a cylinder and the proper corrections made for magnification.

A mitochondria-cytoplasmic ratio was then obtained by dividing the total mitochondrial surface in square microns by the cytoplasmic volume in cubic microns and multiplying by 100. This figure actually represented the mitochondrial surface per 100 cu. μ of cytoplasm.

In order to check the results obtained in this way, several drawings of the same tubule section were made by each of us, and it was found that the ratios agreed closely.

RESULTS

Figure 1 represents, in a diagrammatic way, the size and distribution of mitochondria in cross-sections of the renal tubule at four different levels. It may be seen that they are of greater diameter and length in the proximal convoluted tubule (fig. 1, a) than in any of the other portions, and that the diameters of the mitochondria in the distal convoluted tubule (fig. 1, d), and the ascending limb of Henle's loop (fig. 1, c) are much the same. The descending limb of Henle's loop (fig. 1, b) contains mitochondria of the least diameter, which are also fewer in number.

The results expressing the relationship between cytoplasmic volume and mitochondrial surface are tabulated in table 1. In several instances it shows the extent of variation which may be expected in animals of the same age belonging to the same litter. The proximal convoluted tubule exhibits a greater mitochondrial surface per unit volume of cytoplasm than any of the remaining segments. The mean of the twenty-five determinations is 192.62, with an absolute deviation of 20.5 and a percentage deviation of 10.6. The determinations for the distal convoluted tubule and the ascending limb of Henle's loop yield figures which are somewhat lower. The mean for the distal convoluted tubule is 144.44. The absolute deviation is 16.27 and the relative deviation is 11.2 per cent. The mean value is equal to 74.9 per cent of the value deter-

mined for the proximal convoluted tubule, or the latter segment of the tubule contains approximately 25 per cent more mitochondrial surface than the distal convoluted tubule. The

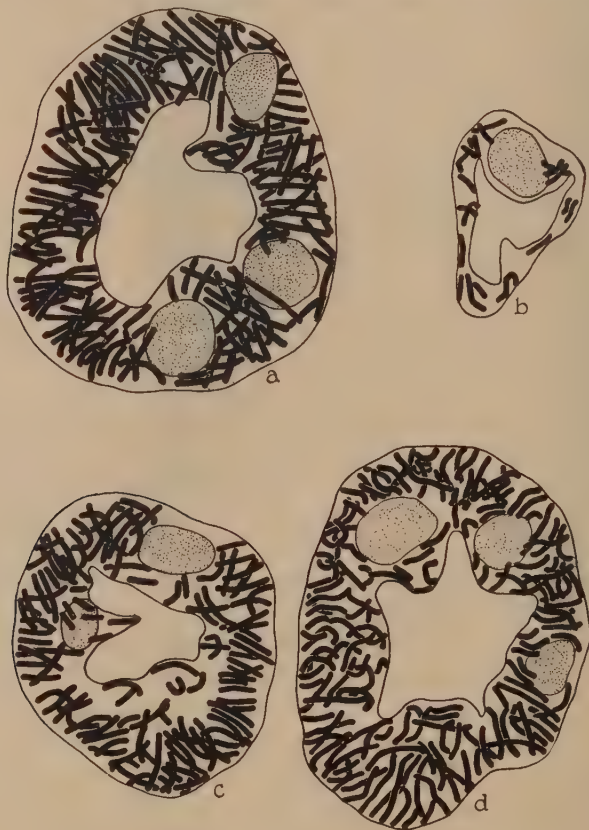


Fig. 1 Mitochondria in cross-sections of the renal tubule. a) Proximal convoluted tubule; b) descending limb of Henle's loop; c) ascending limb of Henle's loop; d) distal convoluted tubule.

differences between the surfaces in the ascending limb and the distal convoluted tubule are not of such magnitude. The mean for the ascending limb of Henle's loop is 138.14 and the absolute and relative deviations are 14.41 and 10.4 per

cent, respectively. The average value for the twenty-five determinations is then 95.6 per cent of the mean value for the same number of estimations in the distal convoluted tubule. This relative figure is close enough to regard the mitochon-

TABLE 1

$$\text{Mitochondria-cytoplasmic ratio} = \frac{\text{Surface of mitochondria}}{\text{Volume of cytoplasm}} \times 100$$

NO.	AGE IN DAYS	SEX	PROXIMAL CONVOLUTED TUBULE	DISTAL CONVOLUTED TUBULE	ASCENDING LIMB OF HENLE'S LOOP	DESCENDING LIMB OF HENLE'S LOOP
R 21	2	f.	188.49	154.13	134.19	79.14
25	14	m.	209.80	113.22	140.44	64.15
4	23	m.	221.51	164.06	159.15	49.87
75	35	m.	235.61	186.36	158.45	52.20
60	42	m.	179.56	118.25	120.69	59.57
29	43	f.	214.98	139.66	130.70	67.14
30	45	m.	154.86	134.58	149.00	66.41
69	45	m.	206.43	150.06	148.81	70.25
70	45	f.	174.31	147.95	142.55	65.35
46	50	m.	174.42	169.65	139.23	52.80
32	50	f.	135.62	187.49	105.05	53.09
1	55	m.	190.55	146.72	158.80	75.09
72	56	f.	195.88	147.05	130.96	56.46
73	68	f.	247.66	139.34	146.94	48.63
11	69	f.	195.07	126.04	119.57	45.54
71	71	m.	204.60	135.80	141.38	80.82
14	72	m.	186.67	126.88	131.14	65.12
74	72	f.	244.32	148.60	178.95	50.85
19	87	f.	193.00	144.31	122.14	48.09
41	95	f.	162.70	121.82	113.37	52.75
13	98	f.	198.89	149.64	128.03	61.48
18	106	m.	153.34	185.05	133.17	67.58
43	125	m.	175.47	112.46	108.56	51.11
44	155	f.	180.37	129.07	138.44	69.36
45	1 yr.	m.	191.44	132.84	173.93	49.94
Mean			192.62	144.44	138.14	60.11

drial surfaces of these segments of the tubule as being practically identical. The mitochondrial surface in the descending limb of Henle's loop is decidedly less than for that of any of the previously mentioned segments. The mean is 60.11, for which the absolute deviation is 8.84, and the relative

deviation is 14.7 per cent. Thus, the mitochondrial surface, per unit volume of cytoplasm, in the descending limb of the loop is 31.2 per cent of the surface for the same amount of cytoplasm in the proximal convoluted tubule, 41.6 per cent for the distal convoluted tubule, and 43.5 per cent when compared with the ascending limb of Henle's loop.

The average of the relative deviations from the mean is larger for the descending limb of the loop, although the absolute departure is less. This probably means that the range for accuracy in estimating the surfaces for the descending limb of Henle's loop is altered by the smaller size of the tubule and the mitochondrial constituents of its cytoplasm.

DISCUSSION

Two observations seem noteworthy: First, that the various segments of the renal tubule do not exhibit the same amount of mitochondrial surface per unit volume of cytoplasm—a fact which is, indeed, revealed by an inspection of the illustrations presented by other workers. Secondly, that the decrease in magnitude of the ratios in a proximodistal direction in the tubule is in the same order as the change in the ratios of the nucleus to cytoplasm (Covell, '26), although the mitochondria-cytoplasmic ratios of the ascending limb of Henle's loop and the distal convoluted tubule appear to be in closer agreement than the nucleocytoplasmic ratios for the same segments.

It is interesting also that no distinct alteration was detected in the mitochondrial surface per unit volume of cytoplasm during postnatal development, from which it would appear that this surface is attained in the renal tubule at some time in the prenatal period, and thereafter is maintained without great change, at least to maturity. What happens in old age cannot as yet be predicted.

There is good reason to believe that the part played by the proximal convoluted tubule in urinary secretion differs radically from that of the other segments. In this portion of the tubule both the nucleocytoplasmic ratio and the mitochondria-cytoplasmic ratio are of the highest magnitude.

CONCLUSIONS

1. The ratio of mitochondrial surface to cytoplasmic volume varies in the different segments of the tubule.

2. It is greatest in the proximal convoluted tubule, practically identical in the distal convoluted tubule and ascending limb of Henle's loop, and least in the descending limb of Henle's loop.

3. The mean values for twenty-five determinations on each portion of the tubule are: 192.62, proximal convoluted tubule; 144.44, distal convoluted tubule; 138.14, ascending limb of Henle's loop, and 60.11 in the descending limb of Henle's loop.

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ON THE FAILURE OF ENDOTHELIAL CELLS, EVEN AFTER DESQUAMATION, TO BE TRANSFORMED INTO WANDERING CELLS, WITH OBSERVATIONS ON THE NATURE OF ENDOTHELIUM

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SIX FIGURES

INTRODUCTION—THE PROBLEM OF THE RELATION OF ENDOTHELIUM TO THE FREE MACROPHAGES

There is still great diversity of opinion as to the question of the origin and genetic relationship of the large mononuclear phagocytes and the various names given to them reflect this divergence. Clasmatocyte, endothelial leucocyte, polyblast, pyrrol cell, histiocyte, reticulo-endothelial cell, are some of the designations which have been applied to the cell which Metchnikoff ('05) called the macrophage, the name which appeals to us as the most satisfactory designation and which will be used in this paper.

One of the groups of problems in regard to the macrophage is concerned with its relation to the endothelium. Does endothelium desquamate or proliferate to form free wandering cells? If it proliferates in this fashion, does the process take place in embryonic stages only or throughout life? Is the formation of wandering cells or leucocytes from endothelium (provided it occurs) confined in the adult to certain regions, such as the spleen or liver, where the endothelium is thought by many to be especially modified, or can it take place anywhere at any time? Do the desquamated endothelial cells, provided they are formed in appreciable numbers, become the monocytes of the blood or the macrophages of the tissues, or are all the large mononuclear wandering cells, wherever

found, 'endothelial leucocytes'? These questions have not been settled.

The theory that endothelium gives rise to all the free phagocytic mononuclear cells was first advanced by Mallory ('98). His evidence was based chiefly upon the presence, in fixed and stained sections from cases of typhoid fever, of mitotic figures in the endothelium and of a 'loosened' appearance of some of the endothelial cells coupled with the finding of an increased number of mononuclear cells in the blood. The bulk of the evidence presented by others who hold to Mallory's theory has depended upon the study of fixed tissues supplemented by the use of vital dyes or injected granular substances, such as carbon suspensions.

An extensive review of the articles dealing with the phagocytosis of carbon and similar substances has been given recently by Foote ('25). There seems to be no doubt of the fact that carbon particles are removed from the circulation within twenty-four hours after injection; that cells containing carbon collect in the sinusoids of the liver, lymph glands, and spleen; and that the cells which take up the carbon are for the most part large mononuclears resembling the cells which stain heavily with vital dyes. The investigators using this method have also found carbon granules lodged in some of the endothelial cells lining the capillaries of certain organs and an occasional mitosis in such a lining cell with carbon granules in its interior. The conclusion is drawn that the carbon granules form a specific stain for the mononuclears of the blood and the endothelial lining cells, and that, therefore, the former are all derived from the latter. However, one of the authors (McJunkin, '19) found that after injections of pure carbon granules, in contrast to India ink and citrated suspensions of carbon, the polymorphonuclear leucocytes of the blood took up the granules in larger proportions than did the mononuclears. The authors (Clark and Clark, '18) found that, after injections of India ink and of carmine granules into the tissue of the tadpole's tail, observation in the living showed that the stellate connective-tissue

cells behaved exactly like the endothelium of the lymphatic capillaries—took granules which came into actual contact with them into the perinuclear areas of the cell, while the wandering cells of the tissues actively migrated toward the injected granules and ingested them in large quantities. Therefore, since such granular material is taken up by different cells under different circumstances, the evidence for the derivation of one class of cell from another, based upon the use of these substances as a specific stain, does not seem to be conclusive.

Many investigators who do not accept the extreme view that endothelium everywhere may give rise to wandering phagocytes consider that there is evidence for such an occurrence in certain specialized organs, such as the liver, spleen, and lymph glands. Thus, the experiments of Evans, Winternitz, and Bowman ('14), on the formation of the tubercle studied with the aid of vital dyes, point to the formation of free wandering cells from the Kupffer cells of the liver, which are generally considered to be endothelial. However, the nature of the endothelium in this class of organs and its relation to the reticulum have never been clearly explained and are very difficult to demonstrate (Downey, '23). Aschoff ('22) has introduced the term 'reticulo-endothelial system' to include the fixed reticular cells of this class of organs and the free phagocytes of the tissues as a functional unit, and Sachs ('26) has recently given an extensive review of the problem from this point of view. The origin of free macrophages from specialized endothelium, even in the specialized regions in which phagocytosis is so conspicuous, cannot be considered as proved, since recent studies of M. R. Lewis ('25) on living cells in tissue cultures raise the question as to whether the Kupffer cells are really endothelium or whether they may not be free macrophages which have lodged in the liver capillaries. And Elliott ('26), in a careful series of experiments, has recently shown that blood from an animal into which powdered carmine has been injected only five minutes previously can be withdrawn, cen-

trifuged, and washed clean of free carmine, and introduced into a second animal where the carmine-containing white blood cells separate out in the spleen, liver, and lymph glands and there become transformed into cells resembling typical 'reticulo-endothelial' cells. In these particular experiments the possibility of the origin of the carmine-containing cells from endothelium would seem to have been effectively eliminated.

Evidence for the desquamation of endothelium, in contrast to proliferation involving mitosis, has also been presented by different investigators. Netousek ('14) and Kryszynski ('16), from experimental studies of animals injected with various bacteria and toxins, found an increase in endothelial-like cells in the blood. The latter investigator found endothelium desquamated in sheets, in the case of a frog which had received iodine injections. Both authors conclude that desquamation of endothelium is merely passive and that it does not occur to any great extent in normal animals, and they saw no evidence for the derivation of mononuclear phagocytes from desquamated endothelium. Recently, Sabin and Doan ('26) have found in smears of normal blood of rabbit and man, stained with vital dyes, cells which they consider to be desquamated endothelial cells. They find such cells present in a variety of forms, ranging from small round cells up to very large phagocytic cells resembling Kupffer cells, and they distinguish this group of cells from the monocytes of the blood. They consider that some of the desquamated endothelial cells are dying cells, while others are actively phagocytic.

Evidence, from studies of the living, in favor of the formation of mononuclear wandering cells and blood cells from endothelium rests on the observations of Herzog ('24) on the tongue of the frog, and of Sabin ('21) on the blastoderm of the chick in cultures. Both of these investigators saw cells move away from the endothelium, both inside and outside the vessels, and become free; many more of them coming from the exterior than the interior. Sugiyama ('26), study-

ing the same material as Sabin, has recently come to the conclusion that it is only the interior surface of the endothelium which gives rise to free cells, and that the exterior wandering cells arise from surrounding mesenchyme cells.

Without going too fully into a detailed discussion of these various studies, we may state that we fail to find indisputable evidence for the definite transformation of an undoubted endothelial cell into a macrophage or monocyte or any other type of free wandering cell. The conclusions drawn from experiments such as India-ink injections are far from inevitable. Herzog's studies on the living were made on the frog's tongue—an object in which it is difficult to see the finest details; while those made by Sabin have already been weakened by her admission that she was in error in describing the formation of clasmatoocytes from the outside of the endothelium.

OBSERVATIONS ON THE RELATION OF ENDOTHELIUM TO WANDERING CELLS IN THE TRANSPARENT FINS OF LIVING AMPHIBIAN LARVAE

During the past eighteen years, one of the authors has been engaged in studies of living cells and tissues in the transparent tail fin of the amphibian larva. This is a region where it is easy to see stellate connective-tissue cells, blood and lymphatic endothelium, and the various types of blood and wandering cells with remarkable clearness and in their normal environment, and where long-continued observations of the same living growing cells and vessels are possible. The tadpoles are anaesthetized with chloretone or urethane and studied in the upright chamber previously described (Clark, '12).

Many of these studies have already been published. The normal growth of individual lymphatic capillaries and of blood vessels was studied, from the first appearance of the vessels in the fin for as long as a month, by continuous observation of the same vessels for from ten to sixteen hours daily, making records of all the endothelial nuclei in the

field (Clark, '09, '12, '18; Clark and Clark, '25). In every case the endothelium of both blood and lymphatic capillaries remained specific throughout the period, increasing by the mitotic division of preexisting nuclei, which remained in every case in the vessel wall and did not become wandering cells. We also studied the endothelium of both lymphatic and blood capillaries and the wandering cells in tadpoles in which minute quantities of foreign substances were introduced into the fin. Lipoid substances (Clark and Clark, '17), starch ('22), protein, agar ('22), paraffin oil ('16), carbon and carmine granules ('18), and croton oil ('20) were some of the substances used. Although daily records of the capillaries of a selected region, including all their nuclei, were made during these experiments, and, in the case of the inflammation produced by croton oil, uninterrupted observations of thirty-six hours were made, no instance of an endothelial cell becoming free and migrating into the blood or outside tissue was ever noted. Experimentally isolated capillaries have also been studied (Clark, '22), and in these cases also the endothelium remained specific and later the isolated vessels united with their respective systems.

Thus from the time when the first lymphatic and blood capillaries invade the fin, the endothelium was found to be specific in its normal growth and under experimental conditions. Not only did these studies of blood and lymph capillaries under a variety of conditions fail to show any instance of the origin of a free cell from endothelium, but the observations all showed, from very early stages, striking differences in behavior between the endothelial cells lining the vessels and the free macrophages. Hence, after years of such intensive study, we have become convinced that, whether or not endothelium gives rise to wandering cells in certain specialized regions, it cannot be a universal occurrence, otherwise it would take place in the capillaries of the tadpole's tail.

We cannot stress too strongly the necessity for long-continued observations of individual living cells in all studies which involve the derivation and properties of cells. Fre-

quently, during our studies of the development of adventitial cells and of inflammation caused by injected drops of croton oil (Clark and Clark, '25, '20), we saw pictures which might well have been interpreted as proliferation of endothelium to form wandering cells had we encountered them in fixed sections or even in the living if watched for only a short time. We repeatedly observed the mitotic division of endothelial nuclei in blood and lymphatic capillaries and a fixed section of an early phase in such a mitosis might well be mistaken for an endothelial cell preparing to free itself from the vessel wall, but in every such case continuous observation showed that the daughter nuclei remained endothelial nuclei maintaining connection with the endothelial tube. Again, in our studies of inflammation, we often saw stages in the migration of a leucocyte through the vessel wall, or in the temporary adherence of a leucocyte to the interior wall which might easily be interpreted, in sections or in casual observation in the living, as the formation of free cells from endothelium, but in every case intensive and continuous observation disclosed the true nature of the phenomenon. Moreover, in studying the wandering macrophages of the tissues we frequently observed the migration of these large mononuclear cells into capillaries and from capillaries to the outside again. Had the process been stopped at certain intervals, we are confident that the illusion of the formation of 'endothelial leucocytes' would have been striking. The phenomenon described by Herzog of cells adherent to the lining of capillaries which were pushed back and forth by the blood stream and finally became free was duplicated on numerous occasions in our observations of the migration of a leucocyte or wandering cell through the vessel wall and of the temporary adherence of blood cells to the endothelium.

OBSERVATIONS OF THE DESQUAMATION OF ENDOTHELIAL CELLS

Although we have never seen the formation of wandering cells from endothelium, in the course of extensive series of observations of the capillaries, connective-tissue cells, and

wandering cell of the tadpole's tail, made by this intensive method of studying individual cells in the living animal, we have on a few occasions observed the desquamation of an endothelial nucleus with its surrounding cytoplasm, and we shall give a description of these comparatively rare instances.

The first of these cases was seen in a 10-mm. larva of *Hyla versicolor* which had been under observation for ten days with daily records of several blood and lymph capillaries and the surrounding connective-tissue cells and wandering cells. On one day it was observed that the larva was not in good condition—the circulation was sluggish and a few dying connective-tissue cells were present in the field of study. On this same day at the beginning of observation it was noted that a lymphatic capillary, in which all the endothelial nuclei had been followed and their changes of position recorded during the preceding week, showed a changed appearance (figs. 1 to 3). One of the nuclear areas (*N1*) located near the tip of the capillary, no longer showed clearly, but instead there seemed to be a new cell present, inside the capillary lumen. After closer observation with the oil-immersion lens, it became apparent that the peculiar cell inside the lymphatic was one of the endothelial nuclei (*N1*), with a small amount of surrounding cytoplasm, which was just in the act of becoming detached from the vessel wall. The character of the large refractile globules present around the nucleus of this cell made its identification certain, since they were like those found in the perinuclear areas of all the lymphatic nuclei of this specimen, while such globules were not present in any other type of cell in the tail. As the cell was kept under observation, it was seen to become free in the capillary lumen. It was then moved along passively, evidently by the lymph current, in a proximal direction until it reached a constricted

Fig. 1 Record of a living lymphatic capillary from the transparent tail of a larva of *Hyla versicolor*. Made on May 20th. The tadpole was not in a healthy condition. The endothelial nuclear areas are numbered *N1*, *N2*, etc. This record should be compared with figures 2 and 3, which show the same capillary on the two succeeding days. *B.V.*, blood vessel; *Lym.*, lymphatic; Leitz drawing eye-piece, oil immersion. $\times 390$.

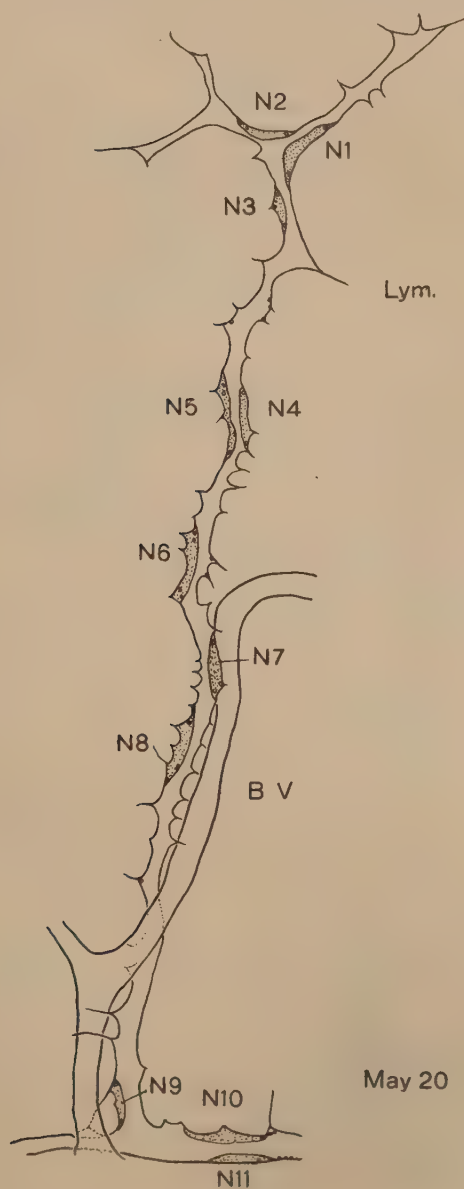


Figure 1

point in the lymph capillary, where it lodged for some time and could be observed in detail (fig. 2, *DE*). This desquamated endothelial cell contained a nucleus of the same shape and size as those remaining in the endothelial wall, with a characteristic sharp outline. The cell was spherical and as it passed along the vessel it rolled over and over, never sending out processes and showing no evidence whatever of independent amoeboid movement. During the day, the cell gradually moved proximally along the capillary, from time to time stopping at the narrow portions. Three hours after the first observation, it was still in the same lymph capillary. At this time its nucleus had become darker in color, more opaque and more distinct, and the cytoplasm showed evidences of degeneration. Seven hours after it became detached from the wall near the tip of the lymphatic, the desquamated cell had arrived at the entrance to the main caudal lymph channel (figs. 1 and 2).

In the meantime another cell of similar appearance was seen in the main caudal lymph vessel. It, too, was rounded, contained a very distinct opaque nucleus and, in the surrounding rim of protoplasm, the characteristic refractile granules of the lymphatic endothelium, and in addition some smaller particles in brownian movement. It was also noted at the end of observation on this day that another endothelial nucleus (*N2*) and its surrounding granular area, located near the tip of the same lymphatic, presented an altered appearance, its cytoplasm having become more swollen and transparent and its nucleus opaque. On the following day, it was

Fig. 2 Series of records of portions of the same lymphatic capillary shown in figure 1, made on May 21st. The endothelial nuclear areas are numbered in the same manner as in the preceding record. It will be seen in the first sketch, 12 noon, that *N1* is no longer to be found in the vessel wall, but, instead, appears as a round cell free in the vessel lumen and is labeled *DE*. (desquamated endothelial cell). The succeeding records, made at intervals during May 21st, show the progress of this cell along the lymphatic capillary as it was passively moved by the lymph current in a proximal direction. At 6.10 P.M., it had reached the entrance to the main longitudinal ventral lymphatic channel. The nucleus in the meantime had become much more opaque and sharply outlined. Leitz drawing eye-piece, oil immersion. $\times 390$.

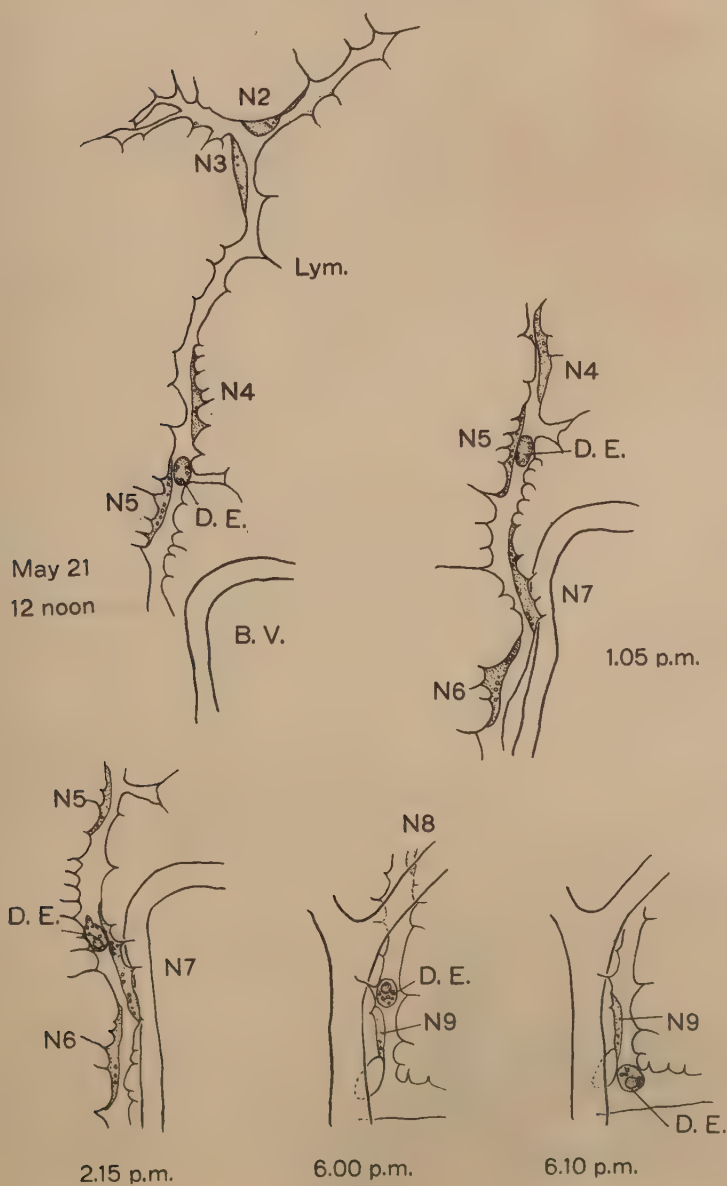


Figure 2

discovered that this nucleus also had disappeared from its former position, while at a point a little farther proximally we found one of the rounded cells with opaque nucleus and characteristic refractile granules, free in the capillary lumen, lodged at one of the constricted points in the vessel (fig. 3, *DE*). Farther posteriorly, two more cells possessing the nuclei and refractile granules characteristic of lymphatic endothelium were observed in the main caudal lymph vessel. They, too, were rounded and showed no sign of independent motility and instead displayed evidence of degeneration in the form of more opaque and clearly outlined nuclei and brownian movement in their cytoplasm (fig. 4). Later, the cytoplasm actually went to pieces inside the vessels, leaving the naked, opaque nuclei.

Of the many specimens in which we have made intensive, continuous daily observation of endothelium and endothelial nuclei, this was the only one in which we found an extensive desquamation of the endothelium. That this evidently exceptional occurrence was probably due to the weakened condition of this particular larva was obvious, since the tadpole was even weaker on the last day of observation and was abandoned as moribund on the day following. The desquamation of endothelium observed in the living animal and just described was in no sense a proliferation of endothelium to form free leucocytes and wandering cells, but instead it was a passive shedding of dead or dying nuclei, with a small amount of surrounding cytoplasm, totally incapable of any independent movement.

In the course of another series of studies we observed an instance of the desquamation of endothelium in a normal healthy larva, which was due to the mechanical pushing of

Fig. 3 Record of the same lymphatic capillary shown in figures 1 and 2, made on May 22nd. The nuclear area marked *N2* in the preceding figures has also disappeared from the capillary wall and appears as a desquamated endothelial cell, *DE*., in the vessel lumen. Another desquamated endothelial cell, distorted in shape, is shown in the main longitudinal lymphatic. The other nuclear areas recorded on the two preceding days may still all be identified. Leitz drawing eye-piece, oil immersion. $\times 390$.

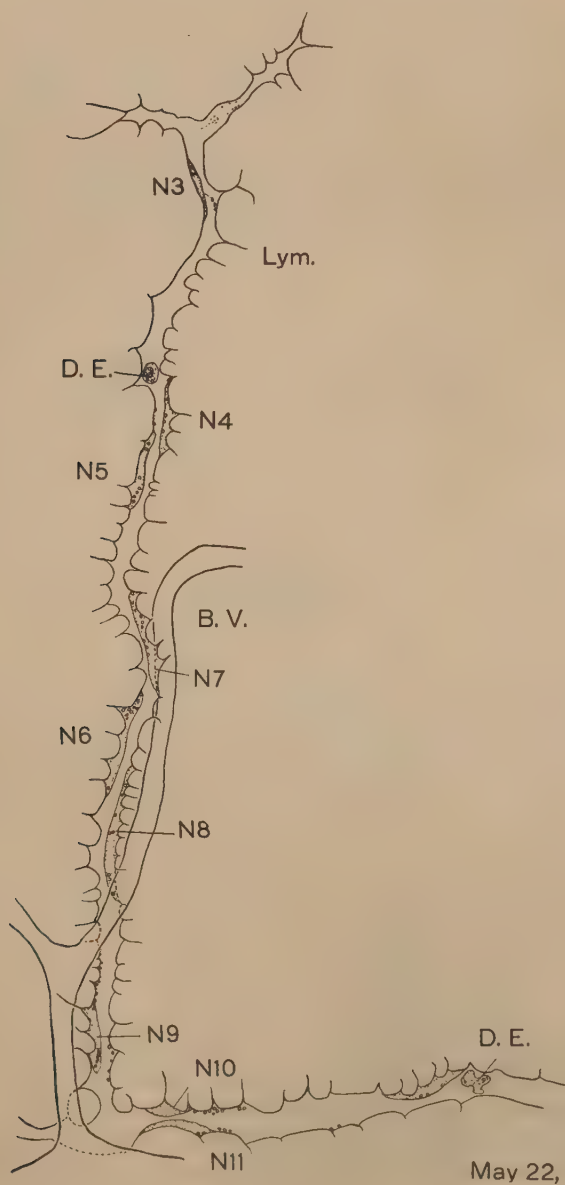


Figure 3

the nucleus from the vessel wall. In this specimen a selected region was under continuous observation for about twelve hours daily. During the preceding week the growth of one of the lymphatic sprouts of this region had been recorded daily. On the day of this particular observation the sprout in question extended for some distance into the fin and contained two endothelial nuclei—one of them containing a large black pigment spot in its perinuclear area (fig. 5, *N1*). The

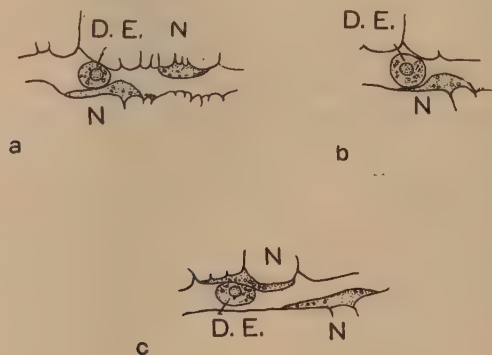


Fig. 4 Records of portions of the main longitudinal lymph vessel in the same tadpole used for the observations recorded in figures 1, 2, and 3. In each case a desquamated endothelial cell is free in the capillary lumen. In all three of these cells the nucleus proper is more distinct and darker than in the uninjured endothelium. In the cell figured in *b*, small arrows indicate the presence of brownian movement in the cytoplasm. *N.*, endothelial nuclear area; *D.E.*, desquamated endothelial cell. Leitz drawing eye-piece, oil immersion. $\times 390$.

pigmented mononuclear macrophages which are in practically constant motion through the tissue spaces of the fin have frequently been observed to migrate through the walls of both blood and lymphatic capillaries. On this day, two such large pigmented wandering cells entered this particular lymph capillary, one proximally, the other near the tip. The macrophage near the tip, after lingering for about an hour inside the capillary lumen, moved out into the tissue again. The proximal macrophage (fig. 5, 8.20 P.M. and 8.30 P.M.) moved in a distal direction, and as this relatively huge cell squeezed

by the region of the two endothelial nuclei, it evidently loosened one of them, for after it had passed, the nucleus (*N1* with the pigment dot) showed signs of injury, becoming more prominent and sharply outlined and showing light and dark areas not visible before. The macrophage continued on its course toward the tip of the vessel and eventually migrated through the wall and back into the outside tissue. A few minutes later, a small non-granular mononuclear cell (fig. 5, *SM*) penetrated the endothelial wall of the same capillary near the tip and moved along the vessel proximally. As it reached the injured nucleus, this small cell was seen to push the nucleus ahead of it, apparently completing its detachment from the vessel wall. The freed nucleus with its perinuclear area became spherical and was carried along by the lymph current in a proximal direction toward the dorsal longitudinal lymphatic. The small wandering cell followed it for some distance, progressing by typical amoeboid movement along the endothelial wall. The desquamated endothelial cell remained rounded and showed no evidence of independent motility. The presence of the dark pigment spot in the perinuclear area facilitated its identification during the time when it remained in view. After lodging for about twenty minutes in a constricted portion of the main lymph vessel, during which time the nucleus became more opaque, it was carried away from the field of observation (fig. 5).

During the course of other observations we have observed an occasional rounded cell with opaque nucleus, and brownian movement in the cytoplasm rolling along passively in the lymph stream which, from the character of the perinuclear granules and the shape of the nucleus, could almost certainly be identified as a desquamated endothelial cell.

In two other instances we were able to follow the process of desquamation by direct observation of the living. Both of these occurred in the same tadpole in the most posterior lymphatic capillary on the day following an injury to the tip of the tail. That the lymphatic capillary was affected by the neighboring injury was evident from its appearance: it be-

came bulbous at the end instead of pointed, and for a short period even had small openings to the exterior, as evidenced by the fact that, with the highest magnifications, some minute pigment granules in the tissue just outside were observed to pass rapidly into the end of the lymphatic and move along inside the lumen.

On the day of the injury to the tail, the desquamation of one of the endothelial nuclei of this lymphatic capillary was observed. The outline of the nucleus became more definite, and two hours later it was free in the lumen of the vessel. In this case a number of macrophages—some of them large enough to fill the capillary lumen—had entered the lymphatic during the day and had moved past this nucleus, and we are unable to state definitely whether one of these large cells had loosened the nucleus in the manner described above (pp. 370 and 371) or whether the desquamation had been due to the effect of the general injury to the whole region.

The other instance occurred in the same capillary on the following day. The injury to the tip of the tail had been largely repaired and the lymphatic had recovered its normal appearance. During this day, too, a number of macrophages

Fig. 5 Series of records from a bullfrog larva, showing actual pushing of an endothelial nucleus and surrounding protoplasm off the wall of a lymphatic capillary by a large pigmented wandering cell (macrophage) which made its way from the outside tissue into the vessel lumen. 8.20 P.M., *N1* and *N2* endothelial nuclear areas (*N1* has a pigment spot which shows in this and the preceding records). *P.W.C.*, pigmented wandering cells. One of them has just entered the lymphatic at the point indicated by the arrow. 8.30 P.M., both pigmented wandering cells are now in the lymphatic. The more proximal one has just moved past the nuclear areas, loosening *N1* in the process. The arrow indicates the point at which the second macrophage entered the vessel. 9.05 P.M., both pigmented wandering cells have migrated out of the capillary and a small mononuclear (*S.M.*), which entered the vessel at the point indicated by the arrow is shown pushing the loosened *N1* ahead of it as it moved actively along the vessel. The following three records show the further passage of the desquamated endothelial nucleus (*N1*) with its surrounding protoplasm, containing the pigment spot, as it was pushed passively along the lymph capillary into the main dorsal lymphatic. 9.20 P.M., *N1* in the main lymph channel. Nucleus proper darker and more distinct. The small mononuclear stuck to the wall of the main vessel and the desquamated cell *N1* rolled along in the lymph stream and was finally carried out of the field of observation. Leitz drawing eye-piece, oil immersion. $\times 390$.

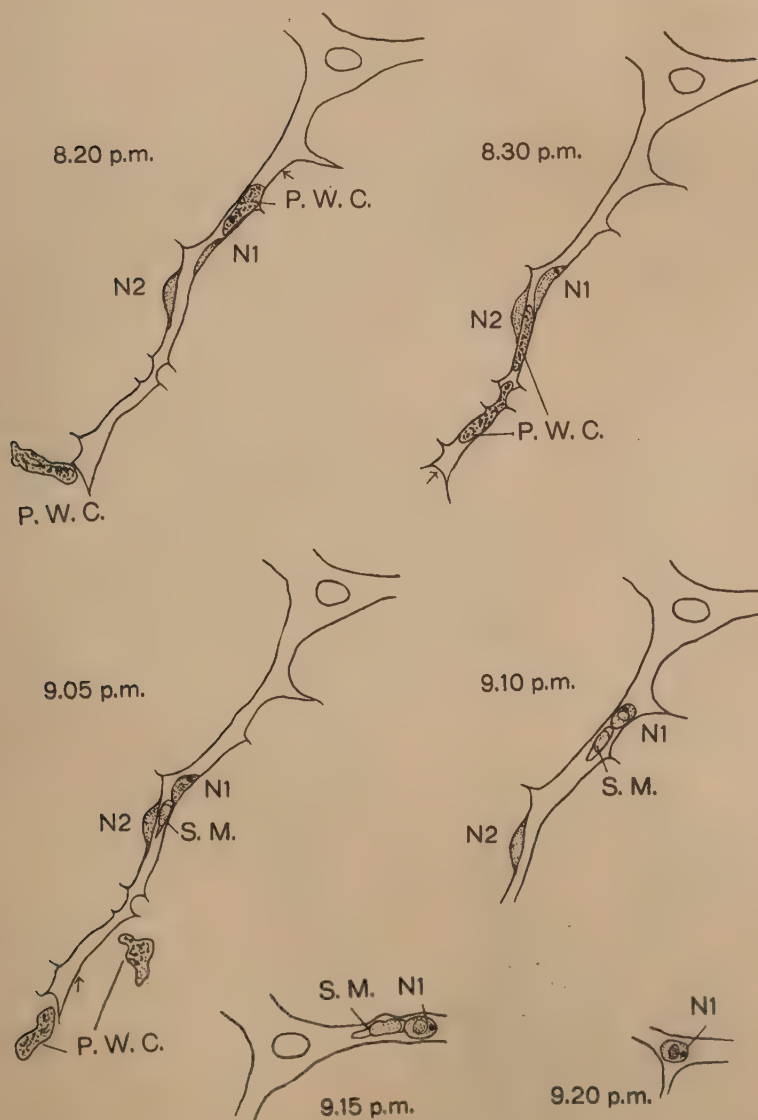


Figure 5

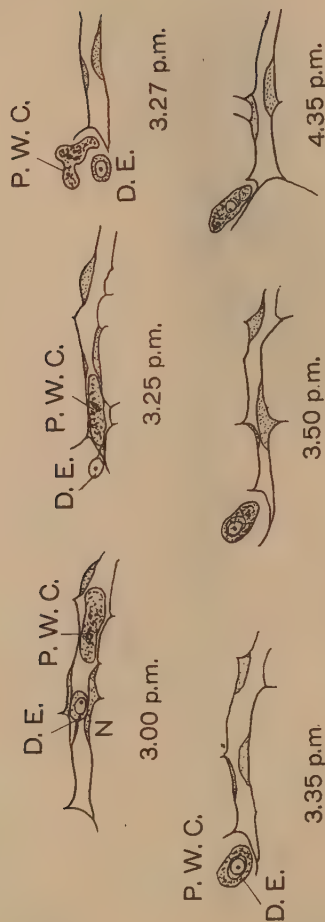


Fig. 6 Series of records taken from a bullfrog larva. A few hours before the first record shown here, several pigmented wandering cells (macrophages) were seen to enter this lymph capillary, to move along the vessel and out again, one of them apparently loosening one of the endothelial cells from the wall. 3.00 p.m., shows one of the pigmented wandering cells, *P.W.C.*, which has recently entered the capillary and is moving along toward the tip. *N.*, endothelial nucleus. *D.E.*, desquamated endothelial cell—still attached to the wall by a small strand of endoplasm. 3.25 p.m., the macrophage has moved along, carrying the desquamated endothelial cell ahead of it, and is shown in the act of pushing it out through the vessel wall. 3.27 p.m., shows both the pigmented wandering cell (*P.W.C.*) and the desquamated endothelial cell (*D.E.*) now outside the lymph capillary. 3.35 p.m., the pigmented wandering cell in the act of engulfing the desquamated endothelial cell. 3.50 p.m., the desquamated endothelial cell is inside the macrophage. 4.35 p.m., the nucleus of the desquamated endothelial cell still shows inside the macrophage. Leitz drawing eye-piece, oil immersion. $\times 390$.

were observed to migrate through the capillary wall, to move along the lymphatic, and to make their way through the endothelium and out again. After the passage of one of these large cells through the portion of the capillary under observation, one of the endothelial nuclei, with its perinuclear area, was observed to be partly separated from the wall and attached to it merely by a thread (fig. 6, 3.00 P.M.). A short time later, another macrophage was seen to enter the lymphatic just proximally to this nucleus and, as it squeezed along the interior of the vessel, it pushed the loosened endothelial cell off the wall and carried it ahead of it. In this case the macrophage continued its way toward the tip of the lymphatic, where it actually pushed the detached endothelial cell through the vessel wall and into the tissue outside. Once outside, the endothelial cell rounded up and remained motionless for a few minutes. Then the macrophage surrounded it and proceeded to ingest it (fig. 6).

The observations of the desquamation of endothelium just described were all made upon lymphatic endothelium. Although blood capillaries have been subjected to the same kind of intensive study for long periods in different seasons, we have not yet seen the process of separation of a nucleus from the wall of a blood capillary. It would appear that desquamation of endothelium is an even rarer occurrence in blood vessels than is the case in lymphatics.

In these observations on desquamation of lymphatic endothelial cells certain points stand out clearly. Whether the cells dropped off or were scraped loose by wandering macrophages, in every case they came loose in the lumen, and not on the outside of the capillary; they became spherical in the lymph, and were moved along passively by the lymph flow, never showing the slightest indication of independent amoeboid movement; their nuclei invariably became opaque, dark, and sharply outlined within about a half-hour after desquamation; brownian movement appeared in the cytoplasm, and, if they remained in view sufficiently long, the cytoplasm completely disintegrated. In the one case in which a macro-

phage pushed a recently desquamated cell through the endothelial wall into the tissue space, it still showed no evidence of amoeboid movement, but, instead, after remaining motionless a few minutes, was phagocytized by the macrophage. Evidently, separation from the endothelial tube was fatal to the nucleus with its surrounding endoplasm. The opportunity to observe the cells for hours after desquamation—which was made possible by the irregular constrictions of the lymphatic capillaries—was an extremely fortunate circumstance, for it enabled us to see the fate of such cells. Moreover, it brings out the excessive caution which must be exercised in such studies, for obviously, in order to prove that leucocytes may arise from endothelium, one must not only see the desquamation, but must watch the desquamated cell long enough to be certain that it has all the properties of a leucocyte.

OBSERVATIONS ON THE STRUCTURE OF ENDOTHELIUM

The present observations appear to have yielded some interesting information in regard to the nature of endothelium. In all the cases of desquamation observed, the endothelial nucleus and its perinuclear area became free on the interior of the capillary, while the exterior wall remained intact. Although the process was observed with the oil-immersion lens, no sign of an opening to the outside tissue appeared at the place at which the nucleus left the wall. This was equally true of the cases in which the desquamation of the nucleus was due to mechanical force—when it was actually pushed off the wall by the large macrophages—as it was of the instance when the process was the result of a degenerative state of the capillary in a dying tadpole.

In this connection, too, a much earlier observation is of interest. A lymphatic was observed in the act of picking up extruded erythrocytes from the tissues and the process recorded (Clark, '09, p. 195, fig. 6). One of the red blood cells after having been transferred to the lumen of the lymphatic sprout was observed to move along proximally, evidently car-

ried by the lymph current. As the erythrocyte moved past one of the endothelial nuclei it was seen to push the nucleus and its surrounding granular area along ahead of it, carrying it some distance from its original position. The blood cell was soon carried past the endothelial nucleus, which then gradually slid back to its former position in the wall. This observation and the instances of desquamation both corroborate the view advanced in an earlier communication (E. R. Clark, '11) that the endothelium of the newly formed capillaries, in amphibian larvae, forms a syncytium. The former evidence given for this concept was the fact that no silver markings can be obtained in young capillaries and the observation that individual nuclei, followed from hour to hour, are seen to shift their relative positions in a vessel and even to move past one another.

All of these observations show that in newly formed endothelium the nuclei are not firmly fixed in position, so that, following injury, they may be desquamated into the lumen without affecting the contour or continuity of the vessel wall, even though that wall consist solely of endothelium. These facts, coupled with the observation that the endothelial nucleus, as it moves along the endothelial tube in its normal growth and on the rare occasions when it becomes separated from the vessel wall, is invariably surrounded by a considerable amount of granular perinuclear material, demonstrate again the existence of an endoplasm and an exoplasm in the endothelium. The basket-work structure of the endoplasm, as shown in flattened total mounts of the fin, fixed in sublimate acetic and examined in 80 per cent alcohol, was described in a former publication (Clark, '11, p. 403). In such a specimen branching protoplasmic threads could be seen connecting one granular perinuclear area with another, while the exoplasm appeared as a uniform homogeneous layer. Somewhat similar endothelial threads can often be seen in living blood capillaries, particularly in those in a constricted condition (Clark and Clark, '25, p. 276). According to our observations, the continuous wall of exoplasm is found on the exterior of the

capillary, while the nuclei, with their surrounding endoplasm, are located on the interior, and any desquamation of endothelial nuclei occurs into the lumen.

Our observations show that the lymphatic endothelium, after its earliest differentiation, is a distinct, specific tissue, which forms in young vessels, a double syncytium consisting of a clear homogeneous exoplasm, which forms a complete outer covering for the endothelial tube, and a basket-like, meshed endoplasm, the latter composed of nuclei, each surrounded by a granular area, which is connected with the granular endoplasm of neighboring nuclear areas by fine protoplasmic threads. The endothelial nuclei and surrounding areas of endoplasm move slowly up or down or circularly in a vessel wall and divide by mitosis as the capillary grows, but their life and physical properties appear to be dependent on their maintaining connection with the rest of the endothelial tissue. If they become dislodged from the endothelial wall the continuity of the exoplasm continues unbroken, but the desquamated nucleus and surrounding endoplasm die.

The endoplasm of the blood capillaries appears to be more closely bound to the vessel wall and, in adult vessels, the connection is probably still more intimate.

CONCLUSION

From years of intensive study of living lymphatic and blood-vascular endothelium, we are convinced that desquamation of endothelial cells is a phenomenon of rare occurrence and that when such desquamation does take place it is due to some abnormal condition of the whole animal or of a region or to mechanical loosening of the nuclei and their surrounding areas of cytoplasm. When such an endothelial cell becomes free in the capillary lumen it is, according to our observations, incapable of independent movement and shows definite signs of degeneration—is, in fact, a dying cell. Thus our observations are in agreement with those of Netoušek ('14) and Kryzinsky ('16), who consider that the presence of endothelial cells in the blood stream is not a normal

occurrence, but due to a passive desquamation caused by injury or disease.

In none of our observations on the normal growth of lymphatic and blood capillaries and on their behavior under experimental conditions, such as inflammation, did we see the slightest evidence for the formation of 'endothelial leucocytes.' Mitotic divisions of endothelial nuclei have been followed repeatedly and records made of the whole process (Clark, '12), but in every case the daughter nuclei remained in the wall of the endothelial tube as typical endothelial cells. We have watched the migration of macrophages and of leucocytes from the tissues into the vessels and also in the reverse direction, but in all our observations endothelium and wandering cells have remained specific and distinct from one another. We therefore disagree with the view of Mallory, Foote, McJunkin, and Permar that mononuclear phagocytes of the tissues and blood stream are constantly being formed from the endothelium. Our studies do not exclude the possibility of such an occurrence in certain specialized regions, but they do bring definite proof that the origin of wandering cells from endothelium is not a universal phenomenon, since it does not occur in the transparent tails of amphibian larvae.

In the studies of Sabin and Doan ('26) on the presence of desquamated endothelium in the normal blood, the authors have classified as of endothelial origin, cells differing widely in size and appearance. It seems probable that the smaller round cells, some of which they describe as showing signs of degeneration, are, in part at least, true desquamated endothelial nuclei with their perinuclear areas, such as those described in the present observations. The largest cells, which they found to contain ingested red cells and debris, would seem to us to be macrophages. Some representatives of this type of cell are, according to our observations, continually making their way from the tissues into capillaries. Also Lewis and Lewis ('25), Carrel and Ebeling ('26), Maximow ('25), and Elliott ('26) have shown that macrophages can develop from

the monocytes of the normal blood. We are unable to identify the other varieties of cells which Sabin and Doan consider to be of endothelial origin with any of those which we have found in *Amphibia*.

We have studied the macrophages of the tissues in the living (Clark and Clark, '26) and plan to give a more complete account of our findings. Although we have traced this type of cell back to early stages, we have seen no evidence for its origin from endothelium. We have as yet no results which bear upon the origin of the monocytes of the blood or upon their relation to the tissue macrophages.

SUMMARY

Numerous observations of living capillaries and wandering cells in the transparent tails of amphibian larvae, where the individual cells with their nuclei can be seen with the greatest clearness and watched for hours and days in the living animal and in their normal environment, have shown no evidence for the formation of wandering cells or of leucocytes from the vessel endothelium, either in normal growth or under experimental conditions. From the stage at which the capillaries first invade the fin, the endothelium and the wandering cells are specific types with totally different physiological properties.

A few instances of the desquamation of endothelial nuclei with their surrounding cytoplasm from the inner wall of lymphatic capillaries were observed in the living animal. The detachment of endothelial nuclei from the inner wall of the capillary occurred as the result either of a weakened condition of the animal or after mechanical injury. The latter was caused, in the cases which we observed, by large wandering cells which migrated from the outside tissue into the capillary and forcibly dislodged the nuclei with their perinuclear areas from the wall. The endothelial cells which became free in this way were never seen to change into wandering cells or leucocytes. On the contrary, they were incapable of independent motion, their nuclei soon became opaque, brownian

movement appeared in their cytoplasm which later disintegrated; in short, they showed all the characteristics of dying cells.

The observations here recorded afford further evidence for the syncytial character of the newly formed capillary endothelium and for the existence of an endoplasm and exoplasm in the endothelial wall.

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THE AGE OF THE ALBINO MOUSE AT NORMAL SEXUAL MATURITY

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ONE FIGURE

The induction of precocious sexual maturity in the rat by daily homeotransplantation of the anterior lobe of the pituitary was reported by Smith ('27), and in the mouse by Smith and Engle ('27 a). A more detailed account is that of Smith and Engle ('27 b). By this treatment sexual maturity in the mouse was induced as early as the fifteenth day of life, following five daily transplants. Ovulation and mating occurred in many of these mice on the weaning date, the twentieth day, following only three daily transplantations of the anterior pituitary.

While this was certainly much earlier than sexual maturity normally occurs in the mouse, the available data on the normal range of age at sexual maturity were insufficient for a satisfactory comparison. Parkes ('25) discussed the first appearance of sexual maturity in both sexes of mice from sixteen litters. The number of female mice in this series is not mentioned, but it is stated that the vaginal orifice was established at the end of the seventh week, and the first ovulation occurred on the fifty-sixth day. However, an analysis of the vaginal smear was not attempted, a histological examination of the ovaries and tubes affording the criteria.

Kirkham ('20), in a brief report, says that "males and females are both sexually mature when about six weeks old."

Because of the failure of these writers to give the range of ages in which a mouse may be expected to become sexually

mature, we have examined a small group from the same colony which has supplied the experimental animals.

This group consists of one hundred female mice, selected at random from litters born during March, April, and May. The animals were weaned at twenty days of age, each group of female litter-mates being ear-marked and kept together in a cage. They were weighed and examined daily from weaning until the establishment of the vaginal introitus. An examination of the vaginal smear in these animals showed, by the appearance of cornified cells, that ovulation usually occurred within twenty-four hours after the vagina opened. The pertinent data for this series are given in table 1. The distribution of ages at sexual maturity is graphically expressed in figure 1, and the distribution of weights at the day on which sexual maturity occurred is shown in table 2.

The range of the time of the opening of the vagina is from the twenty-eighth to the forty-ninth day of life, with the median at the thirty-fifth day. This normal variability contrasts strongly with the uniformity of appearance of sexual maturity in the experimental groups of precociously matured female mice.

The weights of these mice is given in tables 1 and 2, ranging from 10 grams to 16 grams, with a median at 13 grams. The difference between the average weight of the fifty mice maturing earlier than the median and that of those maturing later is less than the probable error in the recorded weight and is of no significance.

Eighty-five of the mice are from twenty-nine litters, with from two to six females in each litter. The data from these animals were analyzed to determine the degree of correlation which might exist between litter-mates, both as to the age at which the vaginal orifice is established and the weight of the animals at this date.

Since the animals were given a serial number at the weaning date, selection being by chance, a scatter diagram was made in which the even-numbered animals were plotted against the odd-numbered animals. From those litters which

TABLE 1

Ages and weights of one hundred albino mice on the day of the establishment of the vaginal orifice. Litter-mates are grouped, with spaces between litters

SERIAL NO.	DAY OF LIFE	WEIGHT IN GRAMS	SERIAL NO.	DAY OF LIFE	WEIGHT IN GRAMS	SERIAL NO.	DAY OF LIFE	WEIGHT IN GRAMS
213	36	13	370	33	13	439	35	11
242	42	14	371	38	15	440	33	12
243	35	12	372	35	14	441	40	11
244	28	13	373	32	14	442	34	14
247	46	12	374	30	13	443	36	12
249	36	10	375	39	14	444	37	13
251	31	12	376	43	13	445	31	14
250	36	13	377	41	12	446	43	15
252	34	11	380	34	12	447	34	13
253	33	12	382	35	12	448	30	12
254	31	14	387	39	13	450	36	14
255	37	13	388	44	13	452	34	13
256	41	14	389	41	12	455	32	11
259	31	13	391	43	14	456	34	14
260	33	14	392	41	12	457	35	15
266	41	13	393	41	14	458	34	13
267	36	11	394	44	13	459	37	14
276	36	13	395	46	14	462	32	14
279	31	13	396	41	13	463	29	14
280	31	11	398	34	16	467	32	13
298	45	11	403	32	12	468	29	14
311	33	12	404	32	12	469	31	12
312	42	16	410	43	14	476	39	12
313	35	15	411	46	14	477	33	13
350	37	13	412	49	15	479	32	14
351	36	14	420	28	14	480	35	13
357	32	11	421	28	15	481	38	12
362	34	14	422	36	16	482	35	14
363	37	13	423	29	16	483	34	12
369	39	13	424	28	13	484	36	14
367	36	14	428	31	13	485	30	12
368	31	13	429	32	13	486	32	13
			431	32	15	488	31	11
			432	32	15	489	42	14

included an uneven number of animals, three or five, one odd- or even-numbered animal was eliminated by lot, and omitted from this calculation.

By a slight modification of the Pearson products moment method, a coefficient of correlation of family resemblance was obtained. For the day of life at the establishment of the vaginal orifice, $r = 0.608 \pm 0.07$; for the weights of the animals at this date, $r = 0.166 \pm 0.11$. A slight family resemblance apparently exists between litter-mate sisters of this group as regards the establishment of the vaginal orifice. Stone ('26) made an analysis of the data from 337 female rats supplied by Dr. H. M. Evans, and found a coefficient of correlation of only 0.22 ± 0.032 for the age of first oestrus in litter-mates. The interval between the opening of the vaginal orifice and the appearance of the first oestrus is only 1.49 days for 325 of the animals studied, so it is legitimate to compare the correlations for the establishment of the vaginal orifice in mice and the appearance of first oestrus in rats. Since the number of mice here reported is considerably smaller than the group of rats analyzed by Stone, the difference may be not greatly significant. The statistical treatment was essentially similar in both cases.

It is not to be expected that in a heterogeneous colony, there would be a high degree of family resemblance between litter-mates in the weight of the animal at the attainment of sexual maturity. That nutritional factors are of importance is recognized, but when all animals of a colony are kept on a uniform diet, the weight differences would be a result of constitutional rather than of environmental factors. Whether racial or geographical factors influence the appearance of puberty in mice, and if so, which is the more important, is not known. Female mice from a colony maintained in Scotland reported by Parkes matured from the forty-ninth to the fifty-sixth day. We have a single case in our group which matured as late as the earliest of these, since we found only one animal which matured on the forty-ninth day, and none later than that. The colony reported on by Kirkham,

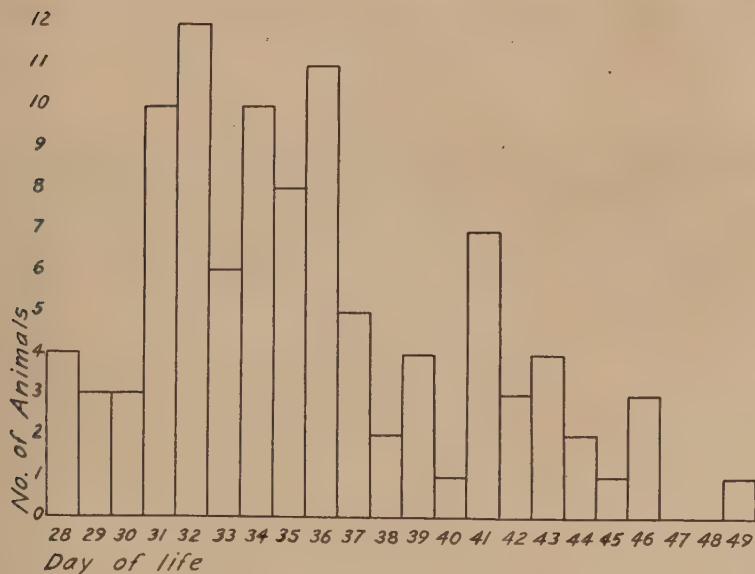


Fig. 1 Distribution of 100 female mice with respect to the age at the establishment of the vaginal orifice.

TABLE 2

Distribution of weights, in grams, of one hundred female mice on day of first appearance of the vaginal orifice

AGE IN DAYS																			
28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
				15															
				15				16											
			14	14		16		14											
			14	14		14		14											
			13	14		14	15	14											
			13	13		14	15	14					14						
			13	13	14	13	14	13					14						
			13	13	13	13	14	13	14				13						
15			12	12	13	13	13	13	13		14		13						
14	16	13	12	12	12	12	12	12	13		13		12	16	14			14	
13	14	12	11	11	12	12	12	11	13	15	13		12	14	14	13		14	
13	14	12	11	11	12	11	11	10	13	12	12	11	12	14	13	13	11	12	15

maintained at New Haven, matured from the forty-second to the forty-ninth day, which falls in our upper range.

SUMMARY

To secure a norm for the first appearance of the vaginal orifice in mice, with the ensuing first oestrus, one hundred mice selected at random have been examined. The range of this first appearance in this series was from the twenty-eighth to the forty-ninth day of life, with a median at the thirty-fifth day. When the coefficient of correlation between litter-mates is determined, $r = 0.608 \pm 0.07$.

The range in weight of the mice is from 10 grams to 16 grams, with a median of 13 grams. There is no greater correlation between litter-mate sisters in respect to the weight on the day of establishment of the orifice than between non-litter-mates.

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SOME INTERESTING ANOMALIES OF THE HUMAN BODY

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THREE FIGURES

The following anomalies were observed in the dissecting-room of the University of Colorado, School of Medicine, and are recorded here, since they not only are interesting morphologically, but also are important from a clinical standpoint. These observations were made over a period of years.

A. MAMMARIA LATERALIS

Three cases. The first case of this anomaly was observed by Doctor Wallin in 1919 in a roentgenological preparation made by him and Mr. Cole. The cadaver from which this preparation was obtained was later used for cross-sections; consequently, no dissections were made. Figure 1 is a reproduction of the roentgenogram and shows this anomaly very clearly.

The other two cases of A. mammae lateralis (Cunningham, '23) or lateral internal mammary (ramus costalis lateralis) (Piersol, '12) were found in 1927, on both sides in one cadaver and on the left side only of another. All three of these vessels were similar in their origin, course, and distribution. These arteries originated from the internal mammary artery just above the first rib and descended upon the inner surfaces of the upper four ribs and the intervening intercostal spaces, parallel with the internal mammary, but about 2 inches lateral to it. In the fourth intercostal space they took a lateral course and terminated by anastomosing with the aortic intercostal artery of that space. During their

course downward they gave off branches in each intercostal space, which anastomosed ventrally with the anterior intercostal branches of the internal mammary and dorsally with the aortic intercostal arteries.



Fig. 1 Cross mark (X) shows the lateral internal mammary artery.

FEMORAL NERVE AND ILIACUS MUSCLE

The condition shown in figure 2 is rather unusual and does not seem to have been described in the literature. It consists of bifurcation of the femoral nerve within the pelvis major. It continues for a short distance as two trunks, after which

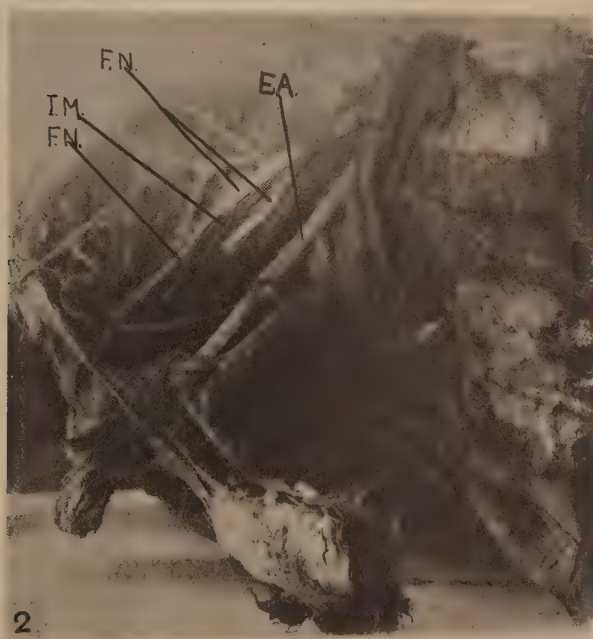


Fig. 2 Femoral nerve and iliacus muscle. *F.N.*, femoral nerve. *I.M.*, portion of iliacus muscle passing through the bifurcated femoral nerve. *E.A.*, external iliac artery.

the two divisions of the nerve reunite to form a single trunk. Through this gap in the nerve passes a portion of the iliacus muscle. The reason for recording this condition is that it may be of interest and importance clinically. When the iliacus muscle contracts during the flexion of the hip-joint it would obviously cause pressure upon the femoral nerve on account of its unusual position, with pain in the area of

its distribution. Such pain in a patient, however, could not be explained by any diagnostic or laboratory methods known. It is also evident that no relief could be effected from such suffering without the knowledge of its cause. But this rare condition, if kept in mind, may help to explain the nature and cause of such pain and, if too troublesome to the patient, could perhaps be relieved either by cutting away that part of the iliacus muscle which passes through the two divisions of the femoral nerve or by dividing that part of the muscle, bringing its two cut ends together posterior to the split femoral nerve and suturing them.

Rare as it may be, this anomaly was found in two cadavers in one year (1924-1925).

M. ADDUCTOR HALLUCIS ACCESSORIUS

This anomaly consists of a muscle in the leg and foot. I have called it *M. adductor hallucis accessorius*. It originates by a fleshy belly from the medial border of the tibia about 3 inches above the medial malleolus below the origin of the flexor digitorum longus for about an inch, and from the deep fascia of the leg.

At its origin it is fleshy, about an inch in width and about 2 inches long, runs obliquely downward and backward superficial to the tibial nerve. At the level of the medial malleolus it becomes tendinous; the tendon passing under cover of the lacinate ligament, lying at first medial to the synovial sheath of the tendon of the flexor hallucis longus and posterior to the tibial nerve. At this point the tendon receives some fleshy fibers about an inch in width, originating from the posterior part of the medial surface of the calcaneus under cover of the origin of the abductor hallucis muscle from the same bone where it dips deeper to the tibial nerve. Beyond this point in the sole of the foot it becomes tendinous again and continues anteriorly up to the level of the inferior surface of the first cuneiform bone, where it becomes fleshy again, and finally unites with the oblique head of the adductor hallucis muscle immediately posterior to its insertion into the head of the first metatarsal bone on its lateral side.

CAROTID ARTERIES

Another interesting anomaly of some clinical significance is that of the carotid arteries shown in figure 3. This was observed in 1925-1926.

On the left side the following condition was found. The common carotid artery bifurcated as usual, but its internal carotid branch was somewhat smaller as compared to the same artery of the right side of the same subject; the external carotid, on the other hand, being somewhat larger than usual.

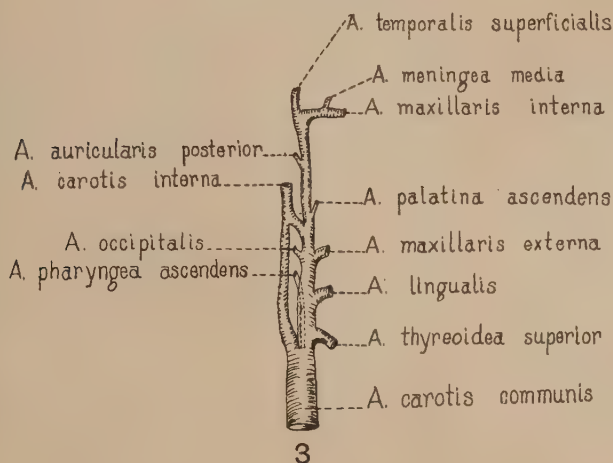


Fig. 3 Free-hand drawing of anomalous carotid arteries.

After giving off the ascending pharyngeal, the superior thyroid, the lingual, the external maxillary, and the occipital branches, the external carotid gave off a branch of considerable size, which anastomosed with the internal carotid artery. Thus the internal carotid attained its usual size beyond this point. The external carotid, after giving off this unusual branch, continued upward in its course and gave off another extra branch, the ascending palatine artery, which is normally a branch of the external maxillary artery, before giving off the posterior auricular and the two terminal branches.

Possibly this anomaly can be explained on the basis of the embryological development of the aortic arches. According to Tandler, quoted by Evans,¹ "the dorsal parts of the second arches become the root portions of the *stapedial artery* on each side." It is possible that the ventral part of the second aortic arch persisted in this case and was responsible for the connecting link between the external and internal carotid arteries.

LITERATURE CITED

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- KEIBEL AND MALL 1912 Manual of human embryology, vol. 2, p. 625. J. B. Lippincott Co., Philadelphia.
- PIERSOL 1912 Human anatomy, p. 764. J. B. Lippincott Co., Philadelphia.

¹ See Keibel and Mall under Literature cited.

BOOKS AND PUBLICATIONS

SURGICAL ANATOMY OF THE HUMAN BODY, by John B. Deaver. Second edition. Three volumes. Size 11 × 8. 2168 pages. 480 plates. Index in each volume. Fabrikoid. Price, \$36.00. P. Blakiston's Son & Co., Philadelphia.

Volume I. Scalp, Cranium, Brain, Face, Mouth, Throat, Organs of Special Sense.

Volume II. Upper Extremities, Neck, Shoulders, Back, Lower Extremities.

Volume III. Joints of the Lower Extremities, Chest, Thorax, Abdomen, Pelvis, Perineum.

In this clinical anatomy Doctor Deaver shares with the medical world the results of his wide experience and incomparable skill. The second edition is revised, enlarged, and brought up to date. The illustrations and the well-arranged text make it possible for the medical man to go over a proposed operation, refreshing his memory as though he were actually seeing the work performed. After a discussion of the superficial anatomy, the different tissues, muscles, nerves, blood vessels, etc., are studied in order as they are met with during the progress of the operation. Particularly are the relations of structures, normal and pathological, stressed. The book teaches the reason and foundation of surgical procedure and explains just what may be done and the dangers to be avoided. Altogether, the set is of value unsurpassed to students, teachers, and practitioners of medicine.

STANDARD METHODS, by Augustus B. Wadsworth. 1927. Cloth. Size 6 × 9. 646 pages. Index. 70 illustrations. References. Price, \$7.50. Williams & Wilkins, Baltimore.

This volume, giving the general laboratory procedures and the methods used in the New York Department of Health, Division of Laboratories and Research, is a guide to practically all the work of the medical technician. Its purpose is threefold: to keep uniform the established methods; to instruct the new worker and prescribe exact procedures for him and for those who are unable to take independent responsibility, and to serve as a guide to the policy which the trained, responsible worker may follow or depart from as the immediate situation demands. General bacteriological technique, the use of experimental and test animals, and the preparation of media and glassware are carefully described. The special methods of the diagnostic laboratories, the laboratories of sanitary and analytic chemistry, the antitoxin serum, and vaccine laboratories are given in order, each series forming a unit in itself. The executive offices, research publications, and the library department also have their special methods, which are given in detail. An excellent book representing an enormous amount of work.

BOOKS AND PUBLICATIONS

VOM LIEBES UND SEXUALLEBEN, 2 volumes, by Ludwig Frank. 1926. Size $5\frac{1}{2} \times 8\frac{1}{2}$. 807 pages. Buckram. Price, bound, M. 16.50; unbound, M. 14.40. Georg Thieme, Leipzig.

Reports of Dr. Ludwig Frank's cases given in the form of letters.

ANATOMICAL, PHYLOGENETICAL, AND CLINICAL STUDIES ON THE CENTRAL NERVOUS SYSTEM, by B. Brouwer. 1927. Cloth. Size $5\frac{1}{2} \times 8\frac{1}{2}$. 84 pages. Illustrated. Bibliographies. Price, \$2.50. The Williams & Wilkins Company, Baltimore.

The Projection of the Retina in the Brain, Pathology of Sensibility, and The Significance of Phylogenetic Studies for the Neurologist. These three studies were given as The Herter Lectures at the Johns Hopkins Medical School in April, 1926. Doctor Brouwer does not limit his discussion to mere facts found by his research work, but adds his own ideas and explanations of the facts. The lectures express the convictions of the author and something of his personality, and the reader may agree or disagree with his interpretations.

THE HUMAN BODY IN PICTURES, by Jacob Sarnoff. 1927. Cloth. Size $8\frac{1}{2} \times 5\frac{1}{2}$. 120 pages. 190 illustrations. Index. Price, \$2.50. Physicians and Surgeons Book Company, Brooklyn.

A visual text of anatomy, physiology, and embryology, designed primarily to be used in connection with motion-picture films and film slides, but complete in itself.

From the foreword: The author illustrates, describes, and correlates the development, structures, and functions of the human body in such a readable and accurate manner as to be understandable alike by the high-school student and the professional aspirant who desires to review the fundamentals of the preclinical sciences. More subjects should be treated in this simple manner, which is not a new form of presentation, but is so well exemplified in this little book.

Tenth International Congress of Zoology

BUDAPEST, SEPTEMBER 4 to 9, 1927

At the Ninth International Congress of Zoölogy, which met at Monaco in March, 1913, it was unanimously resolved to accept the invitation of the Royal Hungarian Ministry of Public Worship and Education to hold the Tenth Congress at Budapest in 1916 under the Presidency of Dr. G. Horváth, Director of the Hungarian National Museum. Owing to the war, it was impossible to carry out this decision, and the Tenth Congress had to be postponed. The international situation has meanwhile so greatly improved that there is no longer any reason for further delay. We have, therefore, the honor to inform you herewith that the Tenth International Congress of Zoölogy will meet at Budapest from the 4th to the 9th of September, 1927, under the patronage of the *International Union of Biological Sciences*, and we cordially invite all zoölogists and those interested in this science to take part in the meeting.

DR. KUNO COUNT KLEBELSBERG, *Privy Councillor*

Hungarian Minister of Public Worship and Education,
representing the Royal Hungarian Government

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President of the Hungarian Academy of Sciences

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General Secretary of the Ninth International
Congress of Zoölogy, representing the Permanent
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Members of the Hungarian Organizing Committee

PRELIMINARY PROGRAM

The program has provisionally been arranged as below. A final and more detailed program will be issued in good time before the Congress. All who wish to take part in the Congress are requested to fill in the forms sent them and to return them as soon as possible to the President of the Congress, Dr. G. Horváth (Hungarian National Museum, Budapest, 80), who will be pleased to give any further information required. The Organizing Committee in Hungary will do everything in its power to arrange for facilities in connection with the journey and the sojourn in Hungary (50 per cent reduction on the Hungarian passport-visa, reduced prices on the railways and in hotels, accommodation in University Colleges, etc.).

Those taking part in the Congress will be either members or associates.

Members paying a fee of 25 pengó (= 18 sh. = \$4.50) are qualified to vote at all meetings of the Congress, to deliver one or more lectures, to bring proposals before the meetings, and to take part in the discussions, receptions, excursions, etc.; they will also receive one copy of the Transactions of the Congress.

Associates paying a fee of 12.50 pengó (= 9 sh. = \$2.25) are entitled to be present at all functions of the Congress, but have no right to vote at the meetings, or to lecture, or to take part in the discussions, nor will they receive the Transactions.

Members and Associates are requested to send their fees by cheque to the Hungarian Commercial Bank of Pest, Branch Belváros (Budapest, IV., Károly-körút 1.) payable to the account of the Tenth International Congress of Zoölogy. The membership card will be sent as a receipt.

The offices of the Congress will be in the Hungarian National Museum.

The formal opening ceremony as well as the farewell meeting and the general meetings will be held in the large hall of the Hungarian National Museum, whereas the sectional meetings will take place in the rooms of the University institutes for natural history and for medicine.

There will be four general meetings and a number of sections, the latter being provisionally arranged as follows:

1. General zoölogy.
2. Experimental cytology (tissue culture).
3. Vertebrata (morphology, physiology, evolution, systematics).
4. Invertebrata (morphology, physiology, evolution, systematics) exclusive of Arthropoda.
5. Arthropoda (morphology, physiology, evolution, systematics).
6. Applied zoölogy (animals useful or injurious).
7. Palaeozoölogy.
8. Zoogeography.
9. Nomenclature.

According to the number of papers announced, each section may be subdivided or several sections may be united.

MEETINGS

Saturday, September 3rd, 8 P.M., the members and associates will be received and entertained by the municipal authorities of Budapest at the Hotel St. Gellért.

Sunday, September 4th

9 A.M. Meeting of the Permanent Committee of the International Congresses of Zoölogy to select for election the vice-presidents, the general secretary of the Tenth Congress, the presidents of the sections, and new members of the Permanent Committee.

11 A.M. Formal opening of the Congress. Addresses. Election of officers and of new members of the Permanent Committee. Introduction of delegates. Organization of the sections. Two lectures of general interest.

4 P.M. Steamer excursion on the Danube.

6 P.M. Landing on St. Margaret's Island.

Monday, September 5th

9 A.M. First general meeting.

3 P.M. Sections.

3 P.M. Meeting of the International Commission on Zoölogical Nomenclature.

7 P.M. Lecture by Dr. Eug. Choinoky, professor, on "Lake Balaton and the Puszta Hortobágy in Hungary (with lantern slides)."

Tuesday, September 6th

9 A.M. Second general meeting.

3 P.M. Sections.

3 P.M. Meeting of the International Commission on the Concilium Bibliographicum.

Wednesday, September 7th

9 A.M. Third general meeting.

3 P.M. a) Visit to the Royal Hungarian Institute of Geology (palaeontological collections), to the Royal Hungarian Museum of Agriculture, and to the Zoölogical Gardens.

b) Excursion to the Svábhegy (Swabian Mountain, 485 meters) and the Jánoshegy (St. John's Mountain, 529 meters) in the neighborhood of Budapest.

Thursday, September 8th

9 A.M. Fourth general meeting.

3 P.M. Sections.

3 P.M. Meeting of the International Commission on Parasitology.

8 P.M. Banquet given at the Grand Hotel Hungaria by the Tenth International Congress of Zoölogy to its members and associates.

Friday, September 9th

9 A.M. Sections.

11 A.M. Meeting of the Permanent Committee of the International Congresses of Zoölogy to consider the place and President of the Eleventh Congress in 1930.

4 P.M. Formal farewell meeting. Report of the General Secretary of the Congress. Selection of the place and election of the President of the Eleventh Congress. Various proposals of general interest. Farewell address of the President.

EXCURSIONS

Arrangements will be made for two excursions to take place after the Congress, one to Lake Balaton and the other to the Puszta Hortobágy.

Saturday, September 10th

Excursion to Lake Balaton and official inauguration of the new building of the Hydrobiological Station of Tihany. (Departure from Budapest in the morning, return in the evening.)

Sunday and Monday, September 11th and 12th

Visit to the Puszta Hortobágy with its herds of horses, cattle, and sheep; inspection of the installation of Pisciculture. (Leave Budapest on September 11th during afternoon for Debrecen, where the night will be spent; next morning by train to the Puszta Hortobágy, where luncheon will be provided; in the afternoon, return to Budapest, arriving in the evening.)

BOOKS AND PUBLICATIONS

AMERICAN ILLUSTRATED MEDICAL DICTIONARY, Fourteenth Edition. Edited by W. A. Newman Dorland. 1927. Octavo of 1388 pages. 319 illustrations, 107 of which are in colors. Flexible binding. Plain \$7.00, with Thumb Index \$7.50. W. B. Saunders Company, Philadelphia and London.

The fourteenth edition includes more than 2000 new terms used in medicine, surgery, biology, dentistry, pharmacy, chemistry, nursing, and kindred branches. The BNA terminology is used throughout and the pronunciation and the derivation of a word is given as well as its definition. Extensive use is made of tables. Under the word "artery" one finds a table listing all the arteries, giving the name, origin, distribution, branches, etc., of each one. Veins, muscles, nerves, poisons, symptoms, tests, operations, exanthemata and many other things are similarly tabulated. The therapeutic table forms a "dose-book" of some fifty pages. Careful selection and arrangement have made a dictionary complete and yet small enough for convenient use. It is the one followed in all The Wistar Institute Publications.

ENZYKLOPÄDIE DER MIKROSKOPISCHEN TECHNIK, III Band. Nachtblau-Zytasen, Sach- und Namenregister. Edited by Rudolf Krause. Third edition. 1927. Size 7×10 . 855 pages, 74 text figures, 19 plates, 14 of which are in colors. Price, bound Mk. 55, unbound Mk. 50. Urban und Schwarzenberg, Berlin and Vienna.

Those scientists who are interested in microscopic technique will be glad to learn that this important reference book, for some time out of print, has been revised, enlarged and brought up to date. It is complete in three volumes. All new and important developments, both practical and theoretical, are incorporated in the new edition. The illustrations are particularly good.

POTASSIUM AND TARTRATES, by Ralph W. Webster and W. A. Brennan. 1927. Size $5\frac{1}{2} \times 7\frac{1}{2}$. 168 pages. Index. Cloth. Price, \$2.50. The Commonwealth Press, Inc., Chicago.

Doctor Webster reviews and sums up the literature treating of the normal occurrence of potassium in the various tissues of the body, and the physiological effects of administering orally and parenterally potassium and the tartrates. The bibliography by W. A. Brennan contains more than two hundred abstracts and references.

THE ROCKEFELLER FOUNDATION, A Review for 1926, by George E. Vincent, President of the Foundation. 1927. 54 pages. Size $9\frac{1}{2} \times 5\frac{1}{2}$. Illustrated.

A brief outline of the work done by the Foundation during the year 1926.

BOOKS AND PUBLICATIONS

FIFTEENTH ANNUAL REPORT OF THE MEDICAL DEPARTMENT OF THE UNITED FRUIT COMPANY. 1926. 356 pages. Size $7\frac{1}{2} \times 10\frac{1}{4}$. Illustrated. Index. The United Fruit Company, Boston, Mass.

A collection of interesting papers dealing with cases, methods, research, and other phases of the company's medical department.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN, Lieferung 236. Edited by Emil Abderhalden. Abt. VII, Methoden der vergleichenden morphologischen Forschung, Heft 3. Stephanie Oppenheim, Adolf Remane, und Wilhelm Gieseler, Methoden zur Untersuchung der Morphologie der Primaten. Mit 69 Abbildungen und 6 Tafeln.

1927 Fortsetzung zu den Lieferungen 57 und 200. Size 7×10 . 131 pages. Price Mk. 9. Urban und Schwarzenberg, Berlin and Vienna.

NEW BOOKS AND PUBLICATIONS

CONDITIONED REFLEXES, An Investigation of the Physiological Activity of the Cerebral Cortex, by I. P. Pavlov. Translated and edited by G. V. Anrep. 1927. Size $6\frac{1}{2} \times 9\frac{1}{4}$. 430 pages, including bibliography and index. Buckram. Price \$9.00. Oxford University Press, American Branch, New York.

In the lectures which make up this book, Doctor Pavlov gives a full and systematized exposition of his twenty-five years' research upon the activities of the cerebral hemispheres of the dog. His point of view and method of approach are purely physiological and purely objective throughout. Starting from Descartes' idea of the simple reflex, he proceeds to the conclusion that there is no essential difference between instinct and reflexes, since both are the inevitable response of the organism to internal and external stimuli. His experiments are based on the reactions associated with the taking of food. Technical methods, formation of conditioned reflexes, varieties of inhibitions, the cortex in normal and pathological states, and the application of his results to man are some of the points he discusses.

RECENT ADVANCES IN PHYSIOLOGY, by C. Lovatt Evans. Second edition. 1926. Size $5\frac{1}{2} \times 8\frac{1}{4}$. 370 pages. 70 illustrations. Cloth. Price \$3.50. P. Blakiston's Son & Company, Philadelphia.

The book is intended to supplement the ordinary text-book in regard to problems of contemporary interest in physiology, and to introduce the reader to important original sources in recent physiological literature. It treats particularly the blood and its circulation, tissue oxidations, muscular contraction, certain endocrines, and postural and conditioned reflexes. It follows the first edition of 1925. The more extensive revision concerns the work of thyroxin and insulin.

PRACTICAL BACTERIOLOGY, BLOOD WORK, AND ANIMAL PARASITOLOGY, by E. R. Stitt. 1927. Eighth edition, revised and enlarged. Size $7\frac{1}{4} \times 5\frac{1}{2}$. 837 pages, including indices. Illustrated with 1 plate and 211 figures. Buckram. Price \$6.00. P. Blakiston's Son & Company, Philadelphia.

A compendium for internists, which contains bacteriological keys, zoological tables, and explanatory clinical notes. In part I, the section devoted to bacteriology, the new bacteriological nomenclature, as presented in the second edition of Bergey's Determinative Bacteriology, and the previously accepted names are both given. Part II, the study of the blood, has been extensively improved, older methods being replaced by more recent procedures, and the sedimentation test discussed at length. A new section, the hemorrhagic diseases, has been added and also a discussion of sickle-cell anemia and glandular fever.

NEW BOOKS AND PUBLICATIONS

Animal parasitology is the general heading of part III, and part IV is called Clinical Bacteriology and Animal Parasitology of the Various Body Fluids and Organs. The appendix contains directions for the more important laboratory procedures, alphabetically indexed. In addition, there is a 25-page index covering the entire book.

GRUNDRISS DER WISSENSCHAFTLICHEN ANATOMIE, by Wilhelm Lubosch. 1925. Size $10 \times 6\frac{1}{2}$. 292 pages. 66 illustrations. Price, bound M. 12, unbound M. 10. Georg Thieme, Leipzig.

A survey of anatomy in its broad biological sense and its relations to other sciences; designed to supplement and round out the usual course in anatomy.

MUSCULAR ACTIVITY, The Herter Lectures for 1924, by Archibald Vivian Hill. 1926. Size $6 \times 8\frac{1}{4}$. 115 pages, 47 illustrations. Bibliography. Cloth. Price \$2.75. The Williams & Wilkins Company, Baltimore.

A series of four lectures delivered at the Johns Hopkins Medical School, summarizing the newer researches in the nature of muscular function, with particular reference to the mechanical, thermal, and chemical aspects. I. Dynamics of Muscular Activity. II. The Heat Production of Muscle. III. Chemical Changes accompanying Muscular Activity. IV. The Recovery Process after Exercise in Man.

STANDARD TESTS FOR REAGENT AND C. P. CHEMICALS, by Benjamin L. Murray. 1927. Second edition, revised and enlarged. Size $6\frac{1}{4} \times 9\frac{1}{4}$. 560 pages, including tables and index. Cloth. Price \$5.00. D. Van Nostrand Company, Inc., New York.

From the preface: "There has been in recent years almost complete lack of agreement as to what quality might reasonably be expected in 'C. P.' chemicals. Consumers, dealers and manufacturers have entertained widely divergent views. The result has been that the expression 'C. P.' has had no definite significance. The standards, together with the equally important methods of testing, herein published, and thus made available to all alike, may serve to diminish this confusion."

NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

A TEXT-BOOK OF HISTOLOGY, Arranged upon an Embryological Basis, by J. L. Bremer. 1927. Size $6\frac{1}{2} \times 9\frac{1}{2}$ inches. 551 pages, including index. 485 illustrations, of which thirty-two are in color. Buckram. Price, \$6.00. P. Blakiston's Son & Co., Philadelphia.

This is a revision of the second edition of Lewis and Stohr's text-book of histology, based on the fifteenth German edition of Stohr's histology. The development of the different structures and their adaptations to special functions are presented in connection with their microscopic anatomy. The opening section on cytology teaches the use of the microscope, the structure of the cell, cytomorphosis, vital phenomena, formation and reproduction of cells. The main part of the volume is made up of general and special histology. A discussion of microscopical technique and the index complete a very excellent text-book.

HANDBUCH DER ANATOMIE DES KINDES, edited by Karl Peter, Georg Wetzel, and Friedrich Heiderich. Zweiter Band, 1. Lieferung. Urogenitalapparat, Sinnesorgane. 1927. Size $6\frac{3}{4} \times 10\frac{1}{4}$ inches. 154 pages. 79 illustrations. Price RM. 24. J. F. Bergmann, München.

A complete modern anatomical treatise designed to fill the need for a reliable representation of the anatomy of the child. It deals with the theoretical and clinical aspects rather than the general subject, and forms a history of part of the development of human anatomy. It is being issued in two volumes, of which only the first section of the second volume has appeared.

LIVING MACHINERY, by A. V. Hill. 1927. Size $6 \times 8\frac{1}{2}$ inches. 306 pages. Illustrated. Cloth. Price, \$3.00. Hareourt, Brace & Co., New York.

Eight lectures delivered at the Lowell Institute, Boston, in March, 1927. They give an idea of the working of two of the most important parts of the body—the muscles which move it about and the nerves which arrange where and how it shall be moved. A combination of human interest and learning makes it a work of exceptional merit both to the reader for pleasure and to the seeker for knowledge.

NEW BOOKS AND PUBLICATIONS

ATLAS DER HISTOTOPOGRAPHIE GESUNDER UND ERKRANKTER ORGANE, by Erwin Christeller. 1927. Size $10\frac{1}{2} \times 12\frac{1}{2}$ inches. 195 pages. 182 colored photographs, grouped to form 88 plates, and 4 text figures. Buckram. Price RM. 90. Georg Thieme, Leipzig.

In the introduction, the author describes his technique for preparing the material and for making the reproductions. The rest of the volume is composed of remarkable colored plates and their explanations.

CANCER CONTROL, 1927. Size $7\frac{1}{2} \times 10$ inches. 336 pages. Cloth. The Surgical Publishing Company, Chicago.

Report of an international symposium held under the auspices of the American Society for the Control of Cancer, Lake Mohonk, New York, September, 1926. The object of the meeting was to take stock of the useful knowledge which exists concerning cancer and the practical ways in which this information can be turned to the greatest possible account. Of the twenty-seven papers presented, fifteen are upon the general topic of organized campaigns against cancer, eight have to do with research, and four with treatment. To lower the appalling death rate, the public must be made to realize the necessity for early and reliable diagnosis. In prompt and proper treatment lies the hope of cure.